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UREA COMPLEXES IN THE SEPARATION OF FATTY COMPOUNDS

By Daniel Swern

ABSTRACT FOR LIST OF PUBLICATIONS

The subject of urea complexes in the separation of fatty compounds is reviewed in detail, under the following main headings: General characteristics; preparation; decomposition and physical properties; applications of urea complexes in fat chemistry; urea complexes in the identification and proof of structure; and miscellaneous applications.

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A. INTRODUCTION

One of the newer separation techniques in organic chemistry and one which has already become an important, widely used and powerful tool in fat chemistry is the formation of crystalline inclusion compounds (also called adducts or complexes) between urea and many straight-chain organic compounds.

Inclusion compounds are combinations of two or more compounds one of which is contained within the crystalline framework of the other. The components of an inclusion compound are each capable of separate existence, and they have no obvious way of uniting chemically. They are held together by secondary valence forces and by hydrogen bonds. Inclusion compounds differ from conventional hydrogen bonded systems, however, in that the size and shape of the "host" and "guest" molecules are critically important in the former but such considerations may play little or no part in the latter.

Inclusion compounds are frequently called adducts or complexes because of the noncovalent nature of the bonds connecting the components and the variation in composition possible with some of them. Urea complexes, however, are true compounds because they contain the two components (host and guest) in definite proportions. They are indistinguishable from ordinary crystalline compounds (with the exception of their decomposition on dissolution or thermal destruction of the framework-forming component, namely, urea), they are isolated and characterized similarly, and their properties differ from those of the components.

Numerous types of inclusion compounds are now known and the subject has been extensively reviewed recently (1-12). Urea complexes are probably the most important class of inclusion compounds, particularly in the fat and petroleum fields. Their properties and uses for separation purposes have been studied more intensively than those of all other types combined.

B. GENERAL CHARACTERISTICS OF UREA COMPLEXES.

In 1940, Bengen (13, 14) discovered that urea forms well-defined crystalline inclusion compounds with straight-chain compounds containing about six or more carbon atoms but not with branched-chain or cyclic compounds. This discovery was an accidental one and arose out of Bengen's research on milk.

Bengen prepared urea complexes from straight-chain hydrocarbons, fatty acids, esters, alcohols, aldehydes and ketones. Water, methanol and ethanol solutions of urea were preferred. Subsequently, it was shown by other investigators, notably W. Schlenk (6, 7), that these and other classes of straight-chain compounds also form urea complexes. Some examples of important classes of straight-chain compounds which form urea complexes are ethers, mercaptans, halides, α,ω-dicarboxylic acids and glycols, 1-monoglycerides, 1,1'-diglycerides, unsaturated hydrocarbons, 1-alkynes nd ketones.

Ease of formation and stability of urea complexes increase with increasing chain length. The upper limit of chain length for formation of urea complexes from various classes of organic compounds has not been determined. The maximum chain length reported to form a urea complex with esters is C_{48} and with hydrocarbons is C_{50}.

In no case studied and confirmed is any urea complex stable above about 133°, the melting point of urea. All urea complexes of the inclusion type apparently have the melting point of urea, although some isolated reports indicate a melting point as high as 142° (15, 16). Most urea complexes undergo decomposition below the melting point of urea. Since the decomposition temperature is characteristic for a complex, it can be used for identification purposes, as is discussed later.

In general, the original principles proposed by Bengen still apply with some exceptions. Although he thought that a minimum chain length of about six carbon toms was necessary for urea complex formation, urea complexes have also been prepared from three-, four- and five-carbon compounds (6, 7, 8). Urea complexes prepared

-rom three-to five-carbon "guest" molecules are quite unstable and must be stored at low temperatures as their survival time at room temperature is low (7). There are few urea complexes which can endure very long at or above room temperature if the chain length of the included compounds is six carbons or less. The rate of increase of half-life time with increasing chain length, however, is so great that at a chain length of eight or more carbon atoms, the stability of urea complexes of straight-chain compounds is usually high. The stability of urea complexes varies in some inverse way with vapor pressure of included species, but low vapor pressure is not the sole criterion for high stability of complex.

Contrary to Bengen's original report, certain branched chain and cyclic compounds will form urea complexes provided there is a sufficiently long straight chain in the molecule and the branch or cycle is not too large (7,7a). 1-Phenyloctadecane and cyclohexyleicosane, for example, in which the rings are at the end of long straight chains, form urea complexes. Phenyl n-octanoate, on the other hand, does not because the straight chain part of the molecule is too short to overcome the effect of the bulky phenyl group; phenyl n-dodecanoate is reported to form a urea complex (17).

The scope and structural limitations of urea complex formation have been extensively studied and described in the literature (1-12, 17). Only that portion pertinent to fat chemistry will be discussed here. A great deal of detailed work attempting to define those principles which will permit a prediction whether a molecule which contains a branch or cycle will or will not form a urea inclusion compounds has been reported by Truter (17) and by Zimmerschied, Dinerstein, Weitkamp and Marschner (18). These investigators examined numerous esters of fatty acids in which the alcohol and/or acid portion were varied in the number and position of branches or cycles. Owing to differences in techniques, there are some discrepancies results and firm guide lines cannot be established as yet. It is unequivocal, however, that a compound reported as a noncomplex former will at best give only very

w yields of complex so that for all practical purposes it is best classified as not forming one. Table I shows the effect of position and number of branches and/or rings on the urea complex forming ability of esters.

Table I can be summarized as follows:

1. All normal esters containing 9-48 carbon atoms form urea inclusion compounds.
2. Each side-chain methyl or ethyl group substituted on the same side of the main saturated chain as the carbonyl oxygen atom requires a straight chain of at least 8 atoms (counting the oxygen atom of the C - O - C group) to enable the compound to form a urea complex. If substitution is on opposite sides of the molecule, at least 11 atoms are required per side group. [Similar conclusions have been reached with methyl-branched hydrocarbons (7a)].
3. A phenyl group on the same side of the main chain as a carbonyl group requires 9 atoms in a straight chain, the larger cyclohexane group requires 13 atoms and the still larger naphthyl group requires 17 atoms to permit complex formation to occur.
4. Benzoates presumably do not form complexes.
5. Two short straight chains are as effective as a long one.
6. The chain length necessary to permit inclusion compound formation depends on the position of the substituent.

One complicating factor in using the above conclusions to determine what separations are practical is that a compound which does not form a complex by itself may form one in the presence of a compound which does. The classic example is the formation of a mixed urea complex from 3-methylheptane and n-octane (7). Also, the converse may occur, namely, a compound that normally forms a complex may be prevented from forming one by a compound that does not (19). These exceptionales occur, however, only when one is operating with compounds which are borderline for forming (or not forming) urea complexes.

Other fat-related compounds which do not form urea complexes are isobutyric and 2-methylbutyric acids (7), the formates, acetates and toluenesulfonates of 12-hydroxystearic acid and of methyl ricinoleate (20), the oximes of ketostearic acid (20), triglycerides (5), cholesteryl esters (17), high-melting 9,10-dihydroxystearic acid, m.p. 131° (21), 9,10-dibromostearic, 9,10,12,13-tetrabromostearic and dibromopalmitic acids (20). However, 9(10)-monobromostearic acid (20) and dibromostearyl alcohol (17) are reported to form complexes.

As is discussed later, branched chain acids or alcohols which do not form urea complexes can be esterified with a suitable straight chain alcohol or acid, respectively, thereby placing the resulting esters in the class of complex formers.

Although urea crystallizes in a tetragonal form, x-ray studies on urea complexes have shown that in its inclusion compounds urea is hexagonal (22). When a urea complex is decomposed by heat the urea reverts to its tetragonal form, but traces of urea of hexagonal shape may survive (23). In forming the complex, urea molecules build up the framework structure in a helical way (6,7,22), so that if one could look down the unit cell one would see a distorted doughnut in which the straight chain organic molecules are able to reside. The unit cell (Figure 1) contains six molecules of urea (solid circles) which occupy the edges of a prism spiralling over a length of 11.1 Å (identity period). The oxygen atoms are located in the edges and the $C\begin{smallmatrix}N\\N\end{smallmatrix}$ groupings lie almost flat in the plane between two adjacent prisms. The diameter of the unit cell is about 8.2 Å, but the size of the channel itself is not explicitly defined.

FIGURE 1

A cross-sectional view of the hexagonal urea is shown in Figure 2 A. The included compound (not shown) occupies the free space inside the hexagonal channels and is attached to the ureas by van der Waals' forces, London dispersion forces or induced electrostatic attractions. The urea channel is about 6 Å at its widest

art and about 5 A at its narrowest. Other values have also been published but the differences are not serious (7, 21, 24).

FIGURE 2

Straight-chain hydrocarbons (Figure 2 B) have a cross section of about 4.1 Å and form urea compounds readily. Hydrocarbons with a single methyl branch (Figure 2 D) require a channel diameter of about 5.5 Å. It can be readily seen why singly branched compounds with a relatively short straight chain, such as 3-methylheptane, do not form a urea complex. Compounds with a double branch on one carbon atom, such as 2,2,4-trimethypentane (Figure 2 E), require a channel diameter of about 6 Å in all directions, and no urea complex has yet been obtained with it and related materials. Benzene (Figure 2 C) requires a channel diameter of 5.9 Å and also does not form a urea complex. As noted previously, when the phenyl group is the end of a long chain, a urea complex forms readily. An explanation for this is that the phenyl group at the end of a chain (phenyl dodecanoate, phenyloctadecane) is restricted from moving with complete freedom and will therefore be in preferred positions for the formation of complex more often than in the case of benzene itself.

In view of the structural requirements for formation of urea complexes, almost all naturally occurring fatty acids, their corresponding n-alkyl esters and alcohols, and many other derivatives form crystalline urea complexes.

PREPARATION OF UREA COMPLEXES

Numerous ways of preparing urea complexes have been published but only those of widest applicability in the separation of fat-derived compounds will be discussed. The common requirement of all the preparative techniques is the achievement of effective contact between urea and the substance (s) to be complexed.

The preparative technique selected depends in part on the solubility and vapor pressure of the compound(s) being studied; whether a high yield or high purity of complex is desired; and, if mixtures are being separated, what degree of separation

wanted. Methanol is the most widely used solvent for preparing complexes; ethanol, isopropanol and water are also often employed (1-12). Other solvent systems reported are petroleum ether (25), benzene and trichloroethylene (26), aqueous acetic acid (27), methyl isobutyl ketone (28), and even liquid ammonia (29). The preparation of urea complexes in the total absence of solvent is rarely employed, although a recently published technique suggests some interesting possibilities (23).

A. PREPARATION OF PURE COMPLEX:

This technique is most useful for preparing complexes from pure compounds when high purity rather than yield of complex is the objective. One gram of organic compound is dissolved in 20 ml. of methanol containing 3 g. of urea. Heat is used, if necessary, to effect solution. If the compound does not dissolve, isopropanol chloroform is added dropwise to the hot solution until it is homogeneous. The solution is allowed to stand for several hours at room temperature or at 0-10°, depending on the chain length of the complex-former and the thermal stability of the resulting complex, and is filtered by suction. It is suggested that a blank experiment be run with 1 g. of compound and 20 ml. of the solvent but with urea absent. Formation of a precipitate in the former but not in the latter case is usually positive evidence of urea complex formation.

B. PREPARATION OF COMPLEXES IN HIGH YIELD:

If it is known a priori or from Procedure A that urea complex formation will occur, a slight modification of Procedure A is recommended if high or quantitative yields (80-100%) of urea complex are desired. The ratio of organic compound: urea: methanol is 1:5:7-20. For many substances, high yields of complex are obtained when the ratio is 1:5:20 but to obtain quantitative yields, the ratio should be e nearly 1:5:7. In the latter case, urea contamination of the complex will occur.

Urea complex formation is an equilibrium process (30, 31)and the large excess of urea employed in Procedure B drives complex formation to completion or nearly so. Procedure B, therefore, is one of the most useful for separating complex-forming substances as an insoluble precipitate from those which do not form complexes. The non-complex formers are isolated from the filtrate (32).

C. PRODUCT DESIRED DOES NOT FORM A UREA COMPLEX:

This procedure is convenient for separating mixtures in which the product sought does not form a urea complex, and also provides more leeway in the choice of a solvent system. Although Procedure C can also be used for preparing complexes, it ordinarily requires a long time for establishment of equilibrium, which may be objectionable.

Five g. of mixture, 15-35 g. of powdered urea, 50-100 ml. of benzene, chloroform, other noncomplex-forming solvent and nonsolvent for urea, and 1-5 ml. of methanol are stirred at room temperature until complex formation is complete. This may require many days for completion depending on the solubility of urea in the solvent-methanol system. Urea is almost insoluble in these solvent systems but is sufficiently soluble and reactive because of the small quantity of methanol to permit complex formation and precipitation, thus setting up a cycle. Noncomplex formers are isolated from the filtrate (33).

By using insufficient urea to form complexes with all the complex-forming components of a mixture, this procedure can also be used in the fractionation of complex-forming substances by one can take advantage of the greater rate of complex formation or the lower solubility of complexes from some of the components.

D. SLURRY TECHNIQUE FOR LARGE SCALE LABORATORY SEPARATION OF MIXTURES:

In this technique just enough solvent, such as methanol, is added to urea to yield a readily stirrable mass. One-fifth to one-seventh as much organic compound as urea is added and stirring is continued until the exothermic reaction, characteristic

f complex formation, is over. At this point, the mixture is filtered and washed with a noncomplex-forming solvent, such as isopentane or isooctane, or with a minimum quantity of cold methanol to remove the noncomplex formers. The time for complex formation varies from about 1-8 hours, depending on the size of the batch and the effectiveness of heat transfer.

This technique cannot be used for the preparation of a pure complex because of urea contamination but it is convenient for large scale use because of the relatively small total volumes to be handled. Procedure D is convenient not only for separating complex-formers from those which do not but also for fractionation of complex-formers by using smaller quantities of urea.

Numerous variations of these basic techniques to suit particular systems have been described, but mainly in patents. The procedures given in detail have been found by the author to be the most useful ones for working with fat-derived materials.

Goldsbrough and co-workers (34) have reported an interesting technique recently. Urea complex formation is conducted in an aqueous phase containing a surface active agent to make the adduct surface hydrophilic thus keeping it suspended and avoiding filtration. Electrolytes are used to avoid emulsions and achieve phase separation.

All of the preparative techniques just described include the use of a solvent, in particular, methanol which is an active solvent for urea. In the absence of a solvent and even with finely powdered urea, complex formation is an extremely slow process. Use of a colloid mill to effect intimate contact of reactants has only slight advantage (35) In a recent publication, Kyriacou (23) showed that urea which had been treated with acetone at room temperature followed complete evaporation of the solvent at 55° retained a small fraction of the urea in the hexagonal form, that is, the form in which urea exists in a complex.

urprisingly, the urea obtained from the decomposition of the urea-acetone adduct formed fatty acid and ester complexes rapidly in the absence of a urea solvent.

The presence of small quantities of hexagonal urea may explain the high-speed formation of complexes reported by Gorin and Rosenstein (36) and Mehta and Murty (37) with the use of so-called "expanded" or "activated" urea. The former investigators (36) reported that urea obtained by decomposing the urea-lauric acid complex in toluene at 110° formed complexes rapidly with cottonseed oil acids in hexane and with tall oil fatty acids. A static urea bed and a cyclic process were employed. The latter (37) reported that "activated" urea, obtained by extracting a urea-fatty acid adduct with benzene, formed urea complexes in petroleum ether without heat or alcohol catalyst.

In working with urea-hydrocarbon adducts, Calderbank and Nikolov (38) concluded that the solvent for urea, usually water or methanol, has no function other than that of precipitating solid urea when its solubility is lowered by mutual solvent action or decrease in temperature. The same separation is achieved when solid urea equal in amount to that precipitated from solution is used in the absence of solvent. With solid urea, however, adduct formation is observable only when the particle size is less than a certain critical value. The kinetics and energetics of urea complex formation with n-octane were also studied by these investigators.

McAdie and Frost (39) have studied the formation and decomposition of the complex between solid urea and n-octane vapor, in the presence and absence of initiators. They concluded that although tetragonal urea of 60-80 mesh is unreactive to n-octane vapor initially, absorption of the hydrocarbon does occur the presence of water vapor. If the hydrocarbon is removed by evacuation, the residual urea is reactive to hydrocarbon and water vapor is now unnecessary.

water vapor, therefore, is needed only to initiate the reaction. Methanol and ethanol vapors are also effective initiators but propanol and ammonia are ineffective. Nitromethane and ethylenediamine are intermediate in activity.

The effect of small amounts of biuret + other minor components in the urea is not clear. Champagnat (40) has reported that 0.2 to 0.5% of biuret in the urea reduces the crystal size of the complex, permitting easier handling, but does not appear to improve the separations. Rigamonti and Riccio (41) indicate that biuret reduces the quantity of adducts formed with fatty acid mixtures, but the adducts are reported to contain more of the saturated fatty acids. Thiosemicarbazide is reported to have no effect, whereas methyl + ethyl urethane lower the yield of complex + monoethyl-urea + guanidine are reported to increase the yield (41a).

It has been suggested (42) that urea complex formation can be inhibited by reduction of interfacial tension. The best inhibitors are reported to be commercial detergents.

Urea complexes may be "recrystallized" from methanol or isopropanol in recoveries usually not exceeding about 60% or from the same solvents one-half to two-thirds saturated with urea in recoveries from 60 - 100%. A urea complex of a fatty compound is assumed to be pure when recrystallization does not change its composition and the weight ratio of urea to included compound is 3:1 (10).

Urea complex formation, as already noted, is an exothermic process. Most of the detailed work on the thermodynamics and equilibria of the processes involved have been described for straight-chain hydrocarbons, but some data are available for fatty acids and esters (7a, 18, 30, 31). Table II lists the heats of formation of urea complexes of some fatty acids, esters, and alcohols. As shown, the molar heat of formation increases with chain length and decreases with introduction of unsaturation. A methyl branch in the chain, as in methyl 12-methyltetradecanoate, causes a sharp decrease in the heat of formation.

Equilibrium constants for the reaction

$$\text{Complex} = \text{Reactant} + m\ \text{Urea}$$

have been determined by Redlich and coworkers (30), Rigamonti and Riccio (31)

and Terres and Sur (7a). According to this definition, the value of K is equal to the mole fraction of the reactant in a perfect solution which is in equilibrium with the complex and solid urea. The lower the value of K, the less complex is dissociated. Table II lists equilibrium constants for fatty acids and esters.

A more convenient expression for preparative and separation work is the reciprocal of K which is a measure of the stability of the complex; these values are also given in Table II. Stability constants increase rapidly with increasing chain length. In the saturated series, the value of the stability constant indicates that it is a relatively easy matter to obtain almost quantitative yields of complex when the chain length of the fatty acid or ester is about eight or more carbon atoms. Progressive introduction of double bonds reduces the stabiility constant by a factor of about ten to twenty per double bond. The values for the complexes of stearic, oleic and linoleic acids are 5000, 480 and 53, respectively; for methyl stearate, methyl oleate, methyl linoleate and methyl linolenate the values are 10,000, 530, 44 and 1.5. As a rough empirical rule, a stability factor of about 20 or higher is needed to obtain high yields of complex.

The stability constant of the complex of elaidic acid is about 1.4 times that of oleic acid (666 compared to 480). It is a well known fact that elaidic acid forms a complex more readily than does oleic acid and the resulting complex has a higher dissociation temperature (43).

For fatty acids, $-\Delta H_{\wedge}$ (kcal./mole) $= 1.654\ n - 0.23$ (where n = the number of carbon atoms); for alcohols, $-\Delta H = 1.54\ n - 6.77$ (7a). Similar expressions have been published for other homologous series of organic compounds, such as paraffins, 1-olefins, and alkyl halides (7a, 18, 30).

42a

ANALYSIS OF UREA COMPLEXES (7, 9, 10)

The composition of urea complexes of fatty compounds is best determined by chemical methods, in particular, functional group analysis. The composition of urea complexes of fatty acids is easily obtained by determining the acid number

the complex. The saponification number permits calculation of the composition of complexes from esters but a slight correction must be made because urea itself has a small saponification number. Complexes of unsaturated fatty compounds can be analyzed by the usual halogen absorption methods. Elemental analysis (C, H, N, etc.) can also be employed and serves as an independent check of the functional group methods.

Alternatively, the complex can be decomposed by addition of water to dissolve the urea or by addition of a hot organic solvent in which the urea is insoluble to dissolve the included fatty compounds. Urea or the included compound(s) can then be determined by any applicable chemical or physical method. Nonvolatile included compounds can be weighed after evaporation of the solvent.

DECOMPOSITION OF UREA COMPLEXES (7, 9, 10)

Since the most widely used application of urea complexes in fat chemistry is in separation procedures, destruction of the complex followed by isolation of the included compounds is of prime importance. The best and simplest method is addition of water to dissolve the urea, leaving the fatty compound(s) as an oil or solid for facile separation. Conversely, heating the urea complex with a non-complexing solvent in which urea is insoluble, such as benzene, isooctane, chloroform or carbon tetrachloride, will extract the included compound from the complex. The latter procedure is infrequently used as it is not as rapid nor as convenient as the water-solution method.

In working with fatty acids or esters a small quantity of hydrochloric acid is advantageous to have in the water to prevent emulsion formation from traces of ammonium soaps.

COMPOSITION AND PHYSICAL PROPERTIES OF UREA COMPLEXES OF FATTY COMPOUNDS

Urea forms solid, well-defined crystalline complexes with fatty compounds in a weight ratio of 3:1. To prepare complexes in high yields it is necessary to employ an excess of urea to drive the equilibrium shown below to completion:

lthough the weight ratio is fixed, the molar ratio of urea increases in a linear fashion with increase in molecular weight of included compound. This is shown in Table III (5, 7, 43, 44), which lists the molar and weight compositions of urea complexes of unsubstituted and substituted fatty acids, methyl esters, n-alcohols, 1-monoglycerides and some miscellaneous compounds. The deviation of the weight ratio from exactly 3:1 stems, in part, from analytical inaccuracies. In most cases, however, where the weight ratio is less than 3:1 the included compound is a relatively volatile one and some loss of included compound occurs during handling. The odor of such urea complexes is characteristic of the included compound. Also, many of these volatile compounds are borderline complex formers and their complexes have low decomposition temperatures. Various investigators have reported the length of urea complexes and their density (6, 7) as well as their x-ray diffraction characteristics (6, 7, 22, 23).

APPLICATIONS OF UREA COMPLEXES IN FAT CHEMISTRY

General: The major application of urea complexes in fat chemistry is in the separation of fatty acids, esters, alcohols and other derivatives from each other or from fats and other noncomplex formers. Other important uses are in the identification or characterization of fat-derived compounds and the storage of readily autoxidizable fatty acids and esters, and various miscellaneous uses to be discussed.

Starting with natural or commercial mixtures, a large number of fatty acids, esters and alcohols have been purified by selective urea complex formation. There are three main types of separation procedures, namely, those based on differences in (a) chain length, (b) unsaturation and (c) branching.

In separations based on chain length differences, advantage is taken of the fact that the longer chain compounds preferentially form urea complexes. If insufficient urea is employed to combine with all the components of a mixture of complex-formers, the longer chain components will combine with the urea and precipitate. For best results, the components to be separated should differ in

.ain length by at least four carbon atoms and preferably by at least six. Obviously when the chain length difference is six or more carbon atoms, other separation methods, such as fractional distillation or crystallization, would ordinarily be preferred. But if the substances are heat-labile or if crystallization temperatures required are inconveniently low, the urea fractionation method, which is usually operative above 0°, may be preferred.

The principle in separations based on differences in unsaturation is that as a long chain fatty compound becomes more unsaturated it shows greater deviation from the normal straight chain structure. At a constant chain length, saturated compounds in a mixture form urea complexes preferentially to monounsaturated, monounsaturated preferentially to diunsaturated, and so on. Taking advantage of this difference in complex-forming ability, purified palmitic (45), oleic (46), linoleic (47, 48, 49), linolenic (47, 50), erucic (51, 52, 53), petroselinic (53, 54) and other fatty acids, as well as their methyl esters, have been isolated from various natural sources. No temperature below 0° is required in these separations as compared to the usual temperatures (as low as -90°) employed in conventional crystallization techniques.

Separation of straight-chain or only slightly branched compounds from the more highly branched has been achieved by preferential urea complex formation (17, 33, 55, 56, 57, 58). An ingenious variation of this approach is to increase the size of a branch and/or the length of a straight chain by chemical reaction thus further enhancing the separability of certain classes of compounds. Thus, both secondary and primary straight-chain alcohols form urea complexes and the difference between their complex-forming ability is only minor because the secondary hydroxyl group is a small branch. Acetylation, however, markedly increases the size of the branch of the secondary alcohols while at the same time increasing the length of the straight chain of the primary alcohols thereby permitting a separation of primary acetates (complex formers) from certain secondary acetates (59) as discussed later.

Another case in which this last technique has been used to advantage is in the purification of ricinoleic acid or its methyl ester in good yield. Although these compounds readily complex with urea, as do most of the other component acids and methyl esters derivable from castor oil, acetylation (60,61) or boration (20) completely prevents complexes from forming with the hydroxyl-containing species thereby permitting a clean separation of the nonhydroxyl components as urea complexes.

The concept of enlarging a branch to interfere with complex formation or of increasing chain length to enhance it is a relatively new concept in urea complexes.

PURIFICATION OF FAT-DERIVED COMPOUNDS

A. OLEIC ACID AND METHYL OLEATE: Schlenk and Holman (62) were the first to show the feasibility of preparing purified oleic acid and methyl oleate by means of urea complexing techniques. These investiators prepared 184 g. of methyl oleate (iodine number, 88.5; 2-3% linoleate, 97-98% oleate, traces of saturates) from 463 g. of the methyl esters of olive oil fatty acids by a multistep process involving a combination of several urea complex separations and fractional distillation. The overall yield of oleate was about 40%.

Swern and Parker (46), on the other hand, starting with the acids or methyl esters from olive oil and using the "single dose of urea" technique, precipitated the complexes of essentially all the saturates and monounsaturates present in the mixture leaving the bulk of the polyunsaturates in the filtrate. One low temperature crystallization of the acids or esters isolated from the complexes and one fractional distillation produced substantially polyunsaturate-free (0.2% or less) oleic acid or methyl oleate of 97-99% purity in 60-65% overall yields.

Similarly, Swern and Parker (63) used various commercial sources of oleic acid, ch as tallow, grease and red oil, as starting materials in isolating purified oleic acid, purity 80-95%. In this procedure, the major part of the saturated acids

is first separated by crystallization from 90% methanol at 0°, followed by addition of urea to the filtrate to precipitate the oleic acid adduct at room temperature.

The experimental procedures described by Swern and Parker (46) for the purification of oleic acid and methyl oleate from olive oil are summarized below.

Purification of Oleic Acid (Solution Method): One thousand grams of olive oil fatty acids (composition: oleic, 80%; linoleic, 3.7%; linolenic, 1%; + saturates, 15%) are dissolved in a boiling solution of 3,600 g. of urea in 9,000 ml. of methanol. Crystals of urea complexes form as soon as the container is removed from the steam bath. The mixture is cooled to 0° overnight and filtered, yielding 3,870 g. of urea complexes. These are stirred with a large volume of hot water to dissolve the urea, yielding 840 g. of almost colorless oil as an upper layer (iodine number, 77; composition: oleic acid, 83.9%; linoleic acid, 0.9%; saturated acids, 15.2%). The yield of oleic acid recovered to this point is 88%. Fractional distillation through a 10-plate column yields 432 g. of a lower, semi-solid fraction, b.p. 192-205°/4 (iodine number, 64.9), and 380 g. of colorless, odorless oleic acid, b.p. 205-206°/3.9 (iodine number, 82.8; composition: Oleic acid, 90.4%; linoleic acid, 0.9%; saturated acids, 8.7%). The overall yield of oleic acid recovered is 43%.

To obtain a higher purity and polyunsaturate-free oleic acid, as well as a better overall yield, the acids isolated from the complex are fractionally crystallized from acetone (12 ml./g.) prior to distillation. From 840 g. of these (iodine number, 77), 140 g. of saturated acids (iodine number, 16) are obtained as a precipitate at -20°; 620 g. of an oleic acid fraction (iodine number, 85; composition: oleic acid, 94.4%; linoleic acid, 0.1%; saturated acids, 5.5%) are obtained as a precipitate at -50°. The yield of oleic acid recovered to this point is 72%. Fractional distillation of the precipitate obtained at -50° yields 500 g. of colorless,

lorless liquid (iodine number, 88.0; composition: Oleic acid, 97.7%; linoleic acid, 0.1%; saturated acids, 2.2%). The final yield of oleic acid is 60%.

Purification of Oleic Acid (Slurry Method): In the slurry method the volume of methanol is reduced to one-half that used in the Solution Method and the time is shorter. In a stainless steel kettle a slurry is prepared consisting of 3,600 g. of urea and 4,500 ml. of methanol. To the well-stirred slurry 1,000 g. of olive oil acids are added at such a rate that the temperature does not exceed 35°. The slurry is stirred until the internal temperature has fallen approximately to room temperature (this requires about 8 hours), and the mixture is filtered. The urea complexes weigh 3,680 g. from which 900 g. of almost colorless oil is obtained (iodine number, 78; composition: oleic acid, 85%; linoleic acid, 1%; saturated acids, 14%). Subsequent processing and results are approximately the same as just described under Solution Method except that yields of oleic acid recovered at each step are about 5% higher.

Purification of Methyl Oleate (Solution Method): Three grams of freshly cut metallic sodium is dissolved in 5,000 ml. of anhydrous methanol. One thousand grams of olive oil are added and the solution is refluxed for 30 minutes. An additional 5,000 ml. of methanol and 3,600 g. of urea are then added, and the mixture is boiled until the urea dissolves. The reaction mixture is cooled to 20° and filtered, yielding 3,400 g. of urea complexes. These are stirred with hot water to dissolve the urea yielding 790 g. of a methyl oleate fraction (iodine number, 71.4; composition: methyl oleate, 81.5%; methyl linoleate, 0.9%; saturates, 17.6%), as an almost colorless upper layer. The yield of methyl oleate recovered to this point is 80%. Fractional distillation yields 170 g. of lower fraction, b.p. 164-179°/4 (iodine number, 50.2), and 550 g. of colorless, odorless methyl oleate fraction, b.p. 180-1°/4 (iodine number, 78.0; composition: methyl oleate, 89%; methly linoleate, 1%; saturates, 10%). The overall yield of methyl oleate recovered is 61%.

Fractional crystallization from acetone (10 ml./g.) of 790 g. of methly oleate (iodine number 71.4), recovered from the urea complexes, yields 140 g. of saturated

ethyl esters (iodine number, 15) at -35°; 580 g. of methly oleate (iodine number, 83.5; composition: methyl oleate, 97.2%; methyl linoleate, 0.2%; saturates, 2.6%) is obtained as a precipitate at -60°. The yield of methyl oleate recovered to this point if 70%. Fractional distillation of the methyl oleate fraction yields 540 g. of methyl oleate, b.p. 184°/4.2 (iodine number, 84.9; composition: methyl oleate, 98.9%; methly linoleate, 0.2%; saturates, 0.9%). The final yield of methyl oleate recovered is 66%.

Purification of Methyl Oleate (Slurry Method): The methanolysis of olive oil is carried out as described earlier, but at its completion no additional methanol is added. The reaction mixture is cooled to room temperature and 3,600 g. of urea are then added. The slurry is stirred until the reaction temperature has again reached room temperature (about 8 hours are required). The mixture is filtered, yielding 3,870 g. of complexes from which 812 g. of methyl oleate (iodine number, 73.4; composition: methyl oleate, 83.8%; methyl linoleate, 1.0%; saturates, 15.2%) is obtained. Subsequent processing and results are approximately the same as just described under Solution Method except that yields of methyl oleate recovered at each step are about 5% higher.

Rigamonti and Riccio (64) have carried out the sodium hydroxide catalyzed alcoholysis of glycerides in the presence of urea, which forms crystalline adducts with the esters as they form. A separate step for isolation of the esters is thereby eliminated.

Other methods of purification of oleic acid and/or methyl oleate in which a urea complex-forming step is involved are given in references 65-70. These reports describe either variations of the procedures given in detail above or urea complex formation is only one of numerous other fractionation steps.

LINOLEIC ACID AND METHYL LINOLEATE:

One of the best and cleancut applications of separations involving urea complexes is the preparation of cis, cis-9, 12- octadecadienoic (linoleic) acid (or its methyl

ethyl ester) in good yield from natural sources rich in this component. Classical bromination-debromination methods for preparing linoleic acid or its esters from linoleic-rich natural sources are known to produce varying amounts of geometric and position isomers, depending on the reaction conditions. Repeated low-temperature crystallization of the reaction products is then required to obtain all cis linoleic acid, with consequent high losses of product. The only other practical methods for obtaining all cis linoleic acid or methyl linoleate are countercurrent solvent extraction, which is applicable both on a laboratory and a large scale (71), and chromatography (72).

Schlenk and Holman (62), without furnishing operational data or product composition, were the first to report that a purified methyl linoleate, iodine number 168 and 173 (calculated, 172.4) could be obtained from corn oil methyl esters in 23% and 14% yields, respectively.

Swern and Parker (47) and Parker, Koos and Swern (48) reported explicit procedures for obtaining concentrates of natural linoleic acid (containing up to 95% of cis, cis-9, 12-octadecadienoic acid) in good yield on a relatively large laboratory scale from safflowerseed and corn oils by a single urea complexing step on the mixed fatty acids or esters. Subsequently, Fore, O'Connor and Goldblatt (49) showed that 98-100% cis-linoleic acid or methyl linoleate could be obtained in a single urea precipitation step by substantially increasing the quantity of urea recommended by Swern and coworkers, although the yield was somewhat reduced.

The principle involved in preparing purified linoleic acid or methly linoleate is that saturates and monounsaturates present in the mixed acids or esters preferentially form urea complexes. If the quantity of urea used is sufficient to precipitate only these two types, linoleic acid or its ester will not complex and is isolable from the filtrate in excellent yield. By the use of larger quantities of urea, thereby sacrificing some of the linoleic acid or linoleate as complex with the saturates and monounsaturates, substantially pure linoleic acid or linoleate is obtained from the filtrate without further segregation procedures.

The experimental procedures of Swern and coworkers (47, 48) and of Fore, O'Connor and Goldblatt (49) for preparing linoleic acid and methyl linoleate are summarized below. The preparation of ethyl linoleate is from unpublished work in the author's laboratory (73).

Purification of Linoleic Acid: To a solution of 880 g. of urea in 2.7 l. of hot methanol, 1 kg. of safflowerseed oil fatty acids (composition: linoleic, 78%; oleic, 12%; saturates, 9.5%) is rapidly added and the mixture is heated and stirred just long enough to effect complete solution. After allowing the solution to cool to room temperature overnight, the precipitate of urea complexes is filtered off and discarded. The filtrate is evaporated to dryness under a stream of inert gas, and several volumes of warm water containing 30 ml. of 6 N HCl is added to it to dissolve the urea. The insoluble oil is dissolved in petroleum ether, hexane fraction, and washed several times with water to remove residual urea and mineral acid. Evaporation of the solvent under an inert gas, the last traces under vacuum at 100°, yields 790 g. of linoleic acid concentrate as a pale amber oil. Vacuum distillation from a Claisen flask yields a forerun boiling at about 155-168°/0.2 mm., which amounts to about 10% of the pot charge, followed by the main fraction boiling at 165-168°/0.2 mm. or 195-199°/1 mm. and n_D^{30} 1.464-1.465. The main fraction, an almost colorless oil which weighs about 610 g., has an iodine number of about 175. Its composition is 95% linoleic acid and 5% oleic plus saturated acids. The yield of linoleic acid is about 75% of that present in the safflowerseed oil used.

Products with an iodine number of 179 (calculated for linoleic acid, 181) are obtained in good yield without difficulty by using 1600 g. of urea and 4000 ml. of methanol for each 1000 g. of safflowerseed oil fatty acids and cooling the mixture to about 5° (49). Otherwise the procedure is exactly as described.

Purification of Methyl Linoleate: Three g. of freshly cut sodium are dissolved in 2 l. of anhydrous methanol. One kg. of safflowerseed oil is added and the solution is refluxed for thirty minutes. Twelve hundred grams of urea and 5 l. of methanol

re then added, and the mixture is boiled briefly to obtain homogeneity. The solution is cooled to 0-5° overnight and the crystalline urea complexes are filtered by suction and discarded. The methyl esters in the filtrate are then isolated as described above under linoleic acid. The methyl linoleate concentrate is a yellow oil weighing 735 g. Fractional distillation yields a small forerun boiling at 180-185°/4mm., followed by the main fraction, boiling at 185-189°/3-4 mm. and n_D^{50} 1.448. The main fraction is a colorless oil weighing 630 g. and has an iodine number of 167. Its composition is 95% methyl linoleate and 5% methyl esters of oleic and saturated acids. The yield is about 85% of the linoleic acid present in the safflowerseed oil.

Purification of Ethyl Linoleate: Alcoholysis of safflowerseed oil is effected with absolute ethanol and sodium as described above. The mixed ethyl esters are separated by dilution of the ethanol solution with 7 l. of warm water containing 30 ml. of 6 N HCl, followed by several water washes to remove excess alcohol, acid and salt. The mixed ethyl esters are dried under moderate vacuum in an inert atmosphere and are then dissolved in 4000 ml. of methanol containing 1600 g. of urea. Heat is applied just long enough to effect complete solution and the system is allowed to cool to 5° overnight. The urea complexes are filtered by suction and discarded. The filtrate is then worked up as described above for linoleic acid. The ethyl linoleate concentrate is a yellow oil which weighs about 525 g. Fractional distillation yields a small forerun boiling at 140-145°/0.1-0.2 mm. followed by the main fraction boiling at 145-150°/0.2 mm. and n_D^{30} 1.456. The main fraction is a colorless oil weighing about 425 g. and has an iodine number of 164 (calculated for ethyl linoleate, 164.5). Its composition is 99+% linoleate. The yield is about 50% of the linoleic acid present in the safflowerseed oil used. (Ethyl linoleate a convenient source of linoleic acid in animal feeding studies.)

Other reports describing the concentration or preparation of purified linoleic acid and/or methyl linoleate from the mixed acids or esters are references 65, 66, 70, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83. Although safflowerseed and corn oils are the most frequently used and are the best and most readily available starting materials, tobaccoseed oil (65), cottonseed oil (76), soybean oil (77, 82) and sesameseed oil (80, 81) have also been employed.

C. LINOLENIC ACID AND ETHYL LINOLENATE:

Perilla oil, which contains about 65% of linolenate, is the best starting material from the standpoint of composition but it is not readily available. Linseed oil, although it contains only about 45-50% of linolenate, is the best all-round source of natural linolenic acid because it is cheap and plentiful.

Application of the urea complex separation technique to linolenic-rich mixtures has been shown by Swern and Parker (47,50) to produce concentrates containing a maximum of only about 80-87% of all cis-linolenic acid or ethyl linolenate. The yield of linolenic component recivered is 55-65%. Although it is possible to precipitate saturated and monounsaturated components with urea almost quantitatively, the difference between the urea complex-forming ability of linoleic and linolenic acids (or their corresponding esters) is too small to permit a clean separation. The major contaminant in linolenic concentrates prepared by urea complex separations, therefore, is the linoleic component, with small amounts of oleic and saturated components.

Purification of Linolenic Acid: To a hot solution of 2,000 g. of urea in 5,000 ml. of methanol, 1,000 g. of linseed oil fatty acids (composition: linolenic, 47%; linoleic, 17%; oleic, 27%; saturated, 9%) are added with good mixing. Immediate precipitation occurs on cooling and the solution is allowed to stand at room temperature overnight. The complexes are filtered by suction and discarded. The bulk of the methanol is evaporated from the filtrate under a blanket of nitrogen and warm water containing a small quantity of hydrochloric acid is added to dissolve the urea. The oil which separates is washed several times with warm water and dried

y heating to a maximum of 100° under vacuum in a stream of nitrogen. The linolenic acid concentrate is an amber oil weighing about 425 g., iodine number 240-245. Distillation from an alembic flask yields 350 g. of linolenic acid concentrate as a pale yellow oil, b.p. 160-2°/0.1; iodine number 253-255 (composition: linolenic, 85%; linoleic, 14%; oleic, 0%; saturates, 1-2%). The yield of linolenic acid recovered is about 65%.

Purification of Ethyl Linolenate: In a hot solution of 4200 g. of urea in 10.6 l. of methanol, 2100 g. of the mixed ethyl esters of linseed oil fatty acids are dissolved. The rest of the procedure is the same as that for linolenic acid. Distillation yields 620 g. of ethyl linolenate concentrate, b.p. 134-8°/0.1; iodine number 230 (composition: linolenate, 82%; linoleate, 12%; oleate, 3%, saturates, 3%). The yield of recovered linolenate is about 55%.

Other reports of the concentration of linolenic acid or ester are references 62, 74, 77, 78, 81, 82, 83, and 84.

D. OTHER FAT-DERIVED COMPOUNDS:

Although the bulk of the published work on the preparation of purified fatty acids and esters is devoted to the oleic, linoleic and linolenic systems just described, palmitic (45), stearic (78), elaidic (79, 85), erucic (51, 52, 53, 83, 86), petroselinic (53, 54), [redacted] ricinoleic (20, 60, 61, 87), α- and β-eleostearic (87a) acids and/or their esters have also been concentrated or purified by procedures involving at least one urea complexing step as an important part of the overall process. With ricinoleic acid, the efficiency of separation and purity of product are improved by acetylation which enlarges the branch, thereby preventing the formation of a urea complex from the acetylated compound (60, 61). Ricinoleic acid of 99% estimated purity is obtained in this way. Alternatively, methyl ricinoleate can be converted to the borate ester, producing a bulky, noncomplex-forming species thus permitting efficient separation of the nonhydroxyl species as urea complexes (20). Elaidic acid forms a urea complex more readily

than does oleic acid, its cis isomer, and can be concentrated from mixtures of the two (88, 89, 90). This is to be expected as elaidic acid resembles stearic acid in its shape, whereas oleic acid is a bulkier molecule.

Purification of Erucic Acid: Shenolikar and Subbaram (53) dissolved 10 g. of the fatty acids from mustardseed oil in a hot solution of 50 g. of urea and 300 ml. of methanol, and allowed the mixture to stand at 18-20° for 12 hours. The complexes were filtered off and about two-thirds of the methanol was evaporated from the filtrate which was again allowed to stand at 18-20° for 12 hours. The final filtrate was treated with slightly acidulated water and the liberated acids were extracted with ether, washed with water and dried. Evaporation of the solvent yielded 4.9 g. of substantially pure erucic acid, m.p. 33° (literature, 33°) and iodine number, 74 (calculated 75).

Similarly, Knafo (52) isolated fairly pure erucic acid from the fatty acids of rapeseed oil. In this procedure, the erucic acid was separated from the mixed fatty acids as a crystalline urea complex, and not from the filtrate.

Purification of Ricinoleic Acid: Knafo (60) acetylated 10 g. of castor oil fatty acids with acetic anhydride-pyridine in the conventional manner and then added the reaction product to 150 ml. of a 40% solution of urea in methyl alcohol. The system was cooled to 0-5° and the urea complexes were filtered and discarded. The filtrate was treated with warm water and extracted with ether. The washed ether solution was evaporated to dryness and the acetylricinoleic acid was saponified with alcoholic potassium hydroxide. Acidification of the resulting soap followed by ether extraction, washing, drying, and evaporation of the ether yielded ricinoleic acid, iodine number, 86; acid number, 190; hydroxyl number, 185, and n_D^{19} 1.4699 (calculated: iodine number 85; acid number, 188 and hydroxyl number, 190; n_D^{20} 1.4697 from literature). The yield of ricinoleic acid was 70%.

Similarly, Mehta and Rao (61) obtained ricinoleic acid of high purity by separating the nonacetylated acids from acetylricinoleic acid using urea in ethanol. These investigators conducted a stepwise precipitation of urea complexes with intermediate evaporation of solvent from the filtrate. They obtained a 64% yield of ricinoleic acid with the following characteristrics: Iodine number, 84; saponification number 185 and n_D^{25} 1.4740 (literature, 1.4703). The purity of this product exceeded that obtained previously by Mehta and coworkers (87) who fractionated the unacetylated acids of castor oil and isolated ricinoleic acid of only 91-95% purity, a value not much greater than the ricinoleic acid content of castor oil fatty acids.

Oleyl alcohol and saturated alcohols have been isolated in good yield from sperm whale oil by selective urea complexing (91, 92). Safflowerseed oil has been converted to the mixed alcohols by sodium reduction, and pure linoleyl alcohol has then been obtained from the noncomplex fraction after precipitation of the saturated and monounsaturated alcohols as complexes (93). Other oils which have been reduced and the resulting alcohols fractionated with urea are sardine, calamary, cottonseed and poppyseed oils (94, 95). It is reported that oleyl alcohol can be isolated from sardine and calamary oils (94), and hexadecanol, oleyl alcohol and linoleyl alcohol from cottonseed and poppyseed oils (95).

Tiedt and Truter (96) and von Rudloff (97) have separated fatty alcohols from the unsaponifiable fraction of wool wax by a urea complexing technique. Approximately 20% of the unsaponifiable fraction of wool wax precipitates as urea complexes, the sterols remaining in the mother liquor. After acetylation of the alcohols isolated from the complex, approximately 20% of them no longer form urea complexes; hence these acetates must have been derived from the secondary alcohol function. The enlargement of the size of the branch by acetylation has now prevented or severely restricted secondary alcohols from forming urea complexes whereas they readily formed complexes as the free alcohols. Thus, a separation of primary from secondary alcohols is possible. This method of separating primary and secondary alcohols has been patented (98).

Acylation is a convenient and practical reaction for lengthening the straight chain of primary alcohols while at the same time enlarging the size of the branch in secondary alcohols. Acetylated primary alcohols give better yields of complexes than do the free alcohols (99).

Caution must be exercised in interpreting the results of separations in which acetylation has been used to enlarge the size of the branch in secondary alcohols. Geiseler and Richter (59) have shown, for example, that the acetates of 2- and 3-octadecanol form urea complexes whereas other secondary acetates do not. Very probably, however, the complex-forming ability of these secondary acetates is considerably less than that of the primary acetate and clean separations can still be achieved by limiting the quantity of urea used. Geiseler and Richter also reported that 2- and 3-octadecanone oximes form urea complexes, whereas the other secondary oximes do not. All of the isomeric normal octadecanols, octadecanones and octadecanal, as anticipated, form urea complexes. Contrary to an earlier report that benzoates are unable to form urea complexes (17), these investigators claim to have made one from 1-octadecyl benzoate (59), a result not entirely unexpected.

Tiedt and Truter (100) separated C_{18}, C_{20}, C_{22}, C_{24} and C_{26} straight chain alcohols from the unsaponifiable fraction of wool wax, utilizing the acylation technique just described. Abe and Watanabe (91) have also utilized this procedure in fractionating the fatty alcohols in sperm whale oil. Umezawa (101), also utilizing urea complexes, investigated the alchols in the unsaponifiable fraction of the oil from the ovaries of the globefish. He identified docosanol, cetyl alcohol, 11-eicosenol and oleyl alcohol, in addition to cholesterol.

ENRICHMENT PROCEDURES

A frequently used application of urea complxes and one which was developed early in the history of the subject is the gross enrichment of materials without isolating a specific compound. In some cases it is difficult, if not impossible,

o distinguish between enrichment and purification, as discussed in the preceding section, and arbitrary decisions have been made in a few cases.

The most obvious application is the separation of mixed straight chain from branched chain or bulky compounds. The principle is to precipitate all urea complex formers from those which do not complex. This is usually accomplished by the use of large quantities of urea to ensure reasonably complete precipitation. In some cases, the complex formers are the desired products, whereas in others the noncomplex formers, isolated from the filtrate, are desired. Occasionally, both fractions are valuable.

Free fatty acids, which readily form urea complexes, have been separated from fats, tall oil, polymerized and oxidized fatty acids, and other groups of noncomplex forming substances.

As several groups of investigators have shown, the free fatty acid content of some natural triglycerides can be reduced to 1% or less by precipitating fatty acids as urea complexes (102, 107). With peanut and olive oils, the free fatty acid content can be reduced to less than 1% as the fatty acid contaminants are efficient complex formers (103). With sardine oil, on the other hand, the free fatty acid content can not be lowered below about 5% even though multiple urea treatment is employed, as the highly unsaturated acids present are not efficient complex formers (105).

Tall oil has been separated into fatty acid-rich fractions by urea complexing (4, 82, 102, 108, 109, 109a). Matsumoto and Tamura (108), for example, using a urea-methanol system segregated tall oil into a fatty acid fraction containing only 4% of rosin acids. The noncomplexing rosin acid fraction, however, contained about 21% of fatty acids. The high content of fatty acids in the rosin acids is to be pected as the main fatty acids in tall oil are diunsaturated and do not complex readily.

Schlenk and Holman (62) were the first to separate unoxidized from oxidized fatty acids by urea complex precipitation of the former in autoxidized soybean oil fatty acids. Oxidation introduces branches in the aliphatic chain which reduces or eliminates the complex-forming ability of these compounds. Furthermore, oxidatively-produced polymers are too bulky to form urea complexes and these also concentrate in the noncomplexing filtrate with the oxidized compounds. This technique was used on a large laboratory scale by Kaunitz and coworkers (110, 111) to concentrate in the noncomplex fraction the methyl esters of polymeric species formed during the autoxidation of lard and cottonseed oil, and of alkyl oleates and linoleates. With oxidized linseed oil fatty acids, however, Catravos and Knafo (112) have reported that the separation of unoxidized from oxidized fatty acids is not highly selective, a situation fully to be expected in such a system. Kaneda, Sakurai and Ishii (113) also applied this separation procedure to autoxidized fatty acids and ethyl esters of linseed oil and were able to effect a concentration of unoxidized (complex fraction) from oxidized-polymerized (noncomplex) products, although the degree of separation was not described.

Coleman, Knight and Swern (32) isolated concentrates of methyl oleate peroxides (peroxide content about 90%) from autoxidized methyl oleate by precipitating unoxidized or only slightly oxidized products as urea complexes, leaving the peroxides in the filtrate (noncomplex). This procedure is applicable on a large laboratory scale and is a convenient one for the preliminary concentration of methyl oleate peroxides from levels of up to about 15% to 90% or higher in a single step. An important feature of the urea complex separation technique is that it is mild, nondestructive and relatively rapid.

Other important uses of the enrichment technique in separating complex-formers from noncomplex-formers are the three- to four-fold concentration of Vitamin A (114), the precipitation of monomeric from polymeric fatty acids in heated vegetable oils (115-122) and the separation of straight chain monomeric fatty acids from cyclized monomeric acids obtained in the thermal polymerization of eleostearic acid and in heated linseed oil

(123, 124). More recently, Friedman and co-workers (125) used molecular distillation and urea complex formation to fractionate ethyl esters prepared from heat-treated cottonseed oil. In this way, linear monomers (complex-forming) were separated from nonlinear (noncomplex-forming) monomers, dimers and higher polymers. The noncomplex-forming materials have high toxicity in animal feeding studies. Perkins and Kummerow (117) have suggested that the proportion of noncomplex-forming material (filtrate) and the molecular weight are more reliable indicators of reduction in the nutritive value of heated edible oils than are iodine numbers. Analytical procedures have been developed for the quantitative determination of urea filtrate acids in such systems (126).

Aylward and Wood (44, 127) have shown that saturated 1-monoglycerides from monocaprylin through monostearin readily form urea complexes whereas 2-monoglycerides do not. The molar ratio of urea to monoglycerides increases linearly with chain length of acyl group but whole numbers are not obtained. Eighty to 90% "monostearin" can be obtained from commercial glyceryl monostearate (40-60% monoglycerides) by urea precipitation (129, 130). Saturated 1, 1-diglycerides also form urea complexes (44, 127, 128, 129, 131) but, as is well known, triglycerides do not.

When the systems are more highly unsaturated or contain glycerides of both short and long chain acids, the separations and enrichments, in general, become relatively poor. Enrichment of monoglycerides from the reaction of glycerol with olive oil (132), soybean and cottonseed oils (133), linseed, sesame and coconut oils (128) have been reported; only poor yields of monoglycerides with a maximum content of monoglycerides of about 80% have been obtained.

When urea-methanol systems are used for the enrichment of monoglycerides, undesirable methanolysis may become an important side reaction. Aylward and Wood (134) have pointed out that methanolysis can be avoided if adduct formation is complete within about thirty minutes. Blanco and Cattaneo (135), on the other hand, have pointed out that methanolysis is negligible when 90-100% methanol is used but is appreciable in refluxing 80% methanol.

Another complicating feature in separating mixtures of monoglycerides is the facile conversion of 2- to 1-monoglycerides during processing (127). A further problem in separations of partial glycerides is the relative complex-forming ability of 1-monoglycerides and 1,1'-diglycerides. Although 1,1'-distearin is reported to complex more readily than 1-monostearin and 1,1'-diglycerides are reported generally to adduct more readily than monoglycerides with the same acyl group (128, 129, 136), the effects of differences in chain length and degree of unsaturation have not been explicitly developed and there is still some disagreement (133).

Heckles and Dunlap (133), for example, studied a series of mono- and diglycerides of saturated and unsaturated fatty acids. They reported that glyceryl monopalmitate, monostearate, and monooleate form urea complexes but the monolinoleate and monolinolenate do not. Also, in mixtures of unsaturated mono- and diglycerides, they concluded that the monoglycerides adduct preferentially. Only slight enrichments of monoglycerides could be obtained from the mixed mono- and diglycerides of cottonseed and soybean oils, a result in contrast to separations attainable in the saturated systems (129, 130).

Heckles and Dunlap (133) concluded that in a mixture containing mono- and diglycerides of saturated and unsaturated fatty acids, urea will separate first on the basis of saturation and second on the basis of degree of esterification of glycerol, conclusions disputed by Mehta and Shah (128).

Moreno and co-workers (137) have reported that the crystal structures of adducts of diglycerides with urea are abnormal. Subsequently (138), they pointed out that diglycerides have one-half their "effective" chain length and this is reflected in lower molar ratios of urea to included compound.

Fatty acid monoesters and diesters of ethylene glycol with C_4-C_{18} fatty acids also form urea complexes (138, 139). Diesters of fatty acids with diethylene

ycol form complexes (140). The molar ratio of urea to ester increases linearly with chain length of acyl group, as observed for monoglycerides (44) and other classes of straight chain compounds.

Another widely used application of urea complexes for enrichment purposes is the segregation of relatively low iodine number from high iodine number fatty acids, methyl esters, alcohols and other derivatives. One urea precipitation is usually employed, taking advantage of the preferential formation of urea complexes by the low iodine number group. One of the most interesting applications of this precedure is the enrichment of fractions of the relatively rare and highly unsaturated fatty acids or esters (four or more double bonds) present in the lipids of various aquatic and land animal tissues.

One of the earliest reports of this enrichment technique is the work of Newey and coworkers (82, 141) who investigated a wide variety of mixtures. Soybean oil fatty acids, iodine number 132, for example, were separated by a single urea precipitation into approximately two equal parts having iodine numbers of 77-90 and 169-177. The major fatty acid in each of these fractions was oleic and linoleic acid, respectively. Reconversion of the linoleic-enriched fraction to triglycerides provided a synthetic drying oil with drying properties equal to that of linseed oil.

Table IV summarizes published work on the enrichment of fatty acids, methyl esters, alcohols and other derivatives from various fats and oils, listed in order of increasing iodine number. As the Table shows, an effective separation of the more saturated from the more unsaturated compounds can be readily achieved. Another noteworthy feature of the enrichment procedure is that the yield of the relatively more saturated fraction precipitated can be regulated by the quantity of urea and solvent employed. Thus, soybean oil fatty acids, iodine number 141, can be separated into pairs of fractions having iodine numbers of 56 and 162, 88 and 180, and 119 and 191 in yields of 9% and 81%, 37% and 56%, and 67% and 27%,

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espectively, as the quantity of urea is progressively increased. Similar results are shown for rice oil and linseed oil fatty acids. As the quantity of solvent (methanol usually) is decreased, the yield of complexes precipitated also increases but selectivity decreases (142).

A striking example of the effect of the ratio of urea to total fatty acids in affecting the yield and iodine number of the fractions was reported by Domart and coworkers (143), who studied the fractionation of menhaden oil fatty acids, iodine number, 159.5, summarized in Table V. By adjusting the urea:fatty acid ratio, from 12-63% of menhaden oil fatty acids can be precipitated as complexes at 1°. Filtrate acids with an iodine number of 193-342 can be obtained in one step, although the yield of the latter material is only 34%.

Abu-Nasr, Potts and Holman (149) have studied the enrichment of fatty acids and esters from various fish oils and of methyl esters from hog adrenal fatty acids, utilizing urea complexing at 5°. Their results are summarized in Table VI. Working on a scale as high as 400 g., these investigators obtained from the noncomplexing filtrate from menhaden oil fatty acids, a fraction with an iodine number of 356, representing concentrates of hexaene acids. With other fish oil fatty acid sources, they obtained fractions with iodine numbers from 294-350. From the methyl esters of hog adrenal fatty acids, final filtrate esters with an iodine number of 190 were obtained (149).

Abu-Nasr and coworkers (149) also studied the enrichment of ethyl and butyl esters of cod liver oil acids and concluded that increased chain length produced by esterification plays a minor role in increasing the yield of included compounds. However, with esters certain polyunsaturated components can be concentrated in the final complex fractions, whereas with the free acids the polyunsaturates are concentrated in the final filtrate (noncomplex).

Using urea complex precipitation as a preliminary concentration and then employing chromatographic separations, Abu-Nasr and Holman (150) were able to

isolate methyl eicosapentaenoate, ethyl docosapentaenoate, and ethyl decosahexaenoate starting with cod liver oil fatty acids. The preliminary urea concentration step produced filtrate acids with an iodine number of 350, starting with acids of iodine number 159.

Silk and coworkers (145, 146, 147) have also utilized urea complex precipitation as a preliminary enrichment technique in the concentration of highly unsaturated fatty acids and alcohols from South African pilchard oil. These investigators showed that the urea complex method is excellent for stepwise fractionation yielding C_{16} and C_{18} concentrates (noncomplex) of high average unsaturation with precipitation of longer chain components of lower unsaturation. Acids were preferred to alcohols for urea fractionation. After a preliminary concentration involving urea complexing, lithium soap separations and molecular distillation, Silk and coworkers used a chromatographic method to isolate hexadeca-6,9,12,15-tetraenoic acid from pilchard oil.

McElroy and coworkers (148) used the enrichment technique on the fatty acids of beef testicular tissue and concentrated fractions rich in docosahexaenoic and arachidonic acids in the complex fractions and a hexaene acid of shorter chain length, probably eicosahexaenoic, in the noncomplexing filtrate.

Whale oil acids have been enriched by Dudkin and Skornyakova (151) who were able to precipitate up to 80% of the acids as urea complexes. The noncomplexing filtrate acids had an iodine number of about 240.

Other reports in which enrichment of natural fatty acids or esters is reported (starting material in parentheses), but usually with less detail than those discussed above, have been published by Achaya and coworkers (152) (peanut, safflowerseed, linseed, cottonseed, castor and dehydrated castor oils); Ericson and Clegg (153) (corn oil); Loury and Heliot (84) (linseed oil); Maselli and Nord (78) (corn and linseed oils and the fat from Fusarium lini); Mehta and Dabhade (80) (sesame

ıd safflower oils); Mehta, Rao and Abhyankar (86) (mustard oil); Mehta, Rao, Lingam, Shah and Prabhu (81) (sesame and linseed oils); Sakurai (142) (rice oil); and Hanson (154) (marine oils), + Rankov + Coworkers (154a) (sunflower oil).

In a recent review, Martinez-Moreno (4) and Schlenk (5) have extensive and convenient tabulations of literature references on enrichment procedures as applied to numberous fats and related naturally-occurring materials.

UREA COMPLEXES IN THE IDENTIFICATION AND PROOF OF STRUCTURE OF FATTY COMPOUNDS

The positive chemical identification of long chain fatty acids, esters, alcohols and related substances is often a problem, as the melting points of derivatives of members of a homologous series are frequently close together. Knight and coworkers (43) have recently developed a method of characterizing a long chain compound which forms a urea complex by determining the dissociation temperature of the adduct.

Although urea complexes have the melting point of urea, Knight and coworkers showed that urea adducts, which are frequently obtained in the form of clear, transparent crystals, become milky and then opaque at a temperature usually below the melting point of urea and characteristic for the particular complex. Experimentally, if a urea complex is slowly heated on a polished copper plate on a Kofler hot stage, while being observed through a low power microscope (24 to 40 X), a temperature will be reached at which milky spots first appear and the entire crystal soon becomes opaque over a temperature range of not more than 1°C, provided the temperature is being increased slowly. The temperature at which milky spots are first observed can be readily duplicated ($\pm$ 1.5°C); this temperature is defined as the "dissociation temperature" of the complex.

Table III lists the dissociation temperatures of urea complexes of saturated, unsaturated and substituted fatty acids, methyl and vinyl esters, alcohols and scellaneous compounds (43) and 1-monoglycerides (44). Adducts of 1-monoglycerides are usually not transparent because of small crystal size. Aylward and Wood (44),

Therefore, consider the temperature at which exudation of molten 1-monoglyceride is noted on the surface of the crystal as the dissociation temperature. In studies with other adducts they showed that this temperature is usually within 1° of the temperature of onset of opacity provided sufficiently small crystals are used.

As Table III shows, dissociation temperatures of the complexes within a homologous series are sufficiently far apart to permit the use of this characteristic for identification purposes. Also, the dissociation temperature increases uniformly with increasing chain length in each homologous series instead of exhibiting the alternation phenomenon so frequently encountered among aliphatic compounds.

The spread in dissociation temperatures observed in the fatty acid series (C_6-C_{18}) is about 60°; in the methyl ester series (C_8-C_{18}) about 75°; in the vinyl ester series (C_9-C_{16}) about 60°; in the alcohol series (C_9-C_{18}) about 65°; in the 1-monoglyceride series (C_8-C_{18}) about 75°. The spread in dissociation temperatures is considerably greater than the usual spread encountered in the melting points of the derivatives ordinarily employed for identification of long chain compounds.

Aside from identification purposes, urea adducts have the additional advantage in permitting facile recovery of the organic compound included. With ordinary derivatives, recovery of the compound being identified is often difficult, if not impossible. Simple addition of water to the complex dissolves the urea and permits separation of the includedcompound from which an additional derivative can be prepared, if desired, for cross checking. In the identification of esters, ease of recovery is important because other chemical techniques for identifying esters require destruction of the ester. Also, the dissociation temperature determination is readily applicable on a micro scale, as all that is required are a few crystals of complex.

As Figure 3 shows, there exists a linear relationship between dissociation temperature and total number of carbon atoms. This relationship is to be expected from the structure of urea complexes (3, 5, 6, 7) which shows that urea associates

n the presence of an aliphatic organic compound to form the walls of a narrow hollow hexagonal cylinder in which the organic compound is held by secondary valence forces. The amount of energy required to break this association should, therefore, depend on chain length, with the van der Waals' forces increasing regularly with each addition of a methylene group.

FIGURE 3

The curves for the five series given in Figure 3 appear to converge between 130 and 140°, and it would appear that the limiting value for dissociating temperature is, in fact, the melting point of urea (133°C). In two isolated cases, however, and not independently confirmed, the melting point of a urea complex has been reported to be as high as 142° (15, 16).

A point of theoretical interest is a comparison of the dissociation temperature of the urea complexes of C_{18} cis-trans isomers (oleic-elaidic acid, methyl oleate-methyl elaidate, oleyl alcohol-elaidyl alcohol). As Table III shows, the dissociation temperature of the trans isomer is significantly higher than that of the cis isomer, although secondary valence forces involved in the formation of complexes from these cis-trans pairs are presumably identical. Examination of molecular models of cis-trans isomers shows that the trans isomers have little or no additional spatial requirements in the urea channel over those of the corresponding saturated compounds. On the other hand, the cis compounds have slightly greater spatial requirements, and slight distortion of the normal shape of the long chain molecule must occur for them to fit within the spiral channel of urea. With the cis compounds, therefore, some steric strain is present, and the complexes should be less stable than those from the corresponding trans isomers. The situation is reflected in lower dissociation temperatures of the urea complexes of the cis isomers.

Another point of theoretical interest is the use of urea complex formation in determining the configurations of the diastereoisomeric 9,10-dihydroxystearic

cids, m.p. 131° and 95°, formed from oleic and elaidic acids, respectively, by hydroxylation with alkaline potassium permanganate. On the other hand, epoxidation of oleic and elaidic acid with organic peracids, followed by hydrolysis of the oxirane ring to α-glycol, yields the 9,10-dihydroxystearic acids, m.p. 95° and 131°, respectively. It is now generally accepted that potassium permanganate hydroxylation of oleic and elaidic acids proceeds by cis addition; peracid oxidation also proceeds by cis addition but opening of the oxirane ring involves an inversion (155). As shown by Swern and coworkers (156), the relative urea complex-forming ability of the two 9,10-dihydroxystearic acids permits an unambiguous confirmation of the stereochemistry of these reactions.

Swern and coworkers (156) calculated the diameters of the two isomeric 9,10-dihydroxystearic acids which would result from cis addition (permanganate hydroxylation), assuming that the normal zigzag structure for both hydroxy acids is maintained. Calculation of the channel diameter required for the formation of a urea complex from the 9,10-dihydroxystearic acid, m.p. 131°, employing Pauling's (157) values for interatomic distances and assuming that its hydroxyl groups are on opposite sides of the chain, gives a value of about 6 Å. Similar calculation of the diameter of the isomer, m.p. 95°, on the assumption that the hydroxyl groups are in the "so-called" cis position, gives a diameter of about 5.4 Å. It is evident, then, that the low melting isomer should form a urea complex readily and in high yield whereas the high melting isomer should either be unable to form a urea complex or should form one in poor yield. The experimental result bears out this conclusion. The low melting isomer gives a urea complex readily in essentially quantitative yield; the high melting isomer does not yield any. Thus, it is shown unambiguously that the high melting 9,10-dihydroxystearic acid has the hydroxyl groups on opposite sides of the chain whereas in the low melting isomer they are substantially on the same side.

The diameter of the high-melting 9,10-dihydroxystearic acid is not too large to permit complex formation to occur under certain conditions. In a 50:50 mixture of the two isomers, some high melting acid is found in the complex but the ratio of low melting to high melting isomer is 3:1. This result also supports the conclusion that the diameter of high melting 9,10-dihydroxystearic acid is significantly greater than that of the low melting isomer.

Urea complexes are formed by the methyl esters of both 9,10-dihydroxystearic acids. The dissociation temperature (Table III) of the complex from the high melting ester is 114° and that from the low melting ester if 120°. This suggests that the complex from the low melting ester is more stable, a result which confirms the conclusion that in the low melting ester the hydroxyl groups are more nearly on the same side of the chain.

For purposes of comparison with fatty compounds of the same number of carbon atoms, the dissociation temperatures of the urea complexes of long chain n-hydrocarbons with urea are as follows: C_{16}, 106-108°; C_{18}, 116-117°; C_{20}, 120-122°; C_{22}, 121-123°; C_{24}, 122-124°; C_{28}, 126-129°; and C_{30}, 130-131° (158). In general, urea complexes of straight-chain, saturated fatty compounds dissociate at higher temperatures than the nonpolar paraffins with the same number of carbon atoms.

STABILIZATION OF FATTY COMPOUNDS AGAINST AUTOXIDATION

A remarkable property of urea complexes is the stabilization of unsaturated fatty acids and esters against autoxidation, an observation first reported by Schlenk and Holman (62) who noted that urea complexes of fatty acids did not become rancid on long term storage. These investigators also compared the oxygen uptake of pure linoleic and linolenic acids and of their adducts, and of free and complexed soybean oil fatty acids over a period of weeks. The urea complexes absorbed

gligible amounts of oxygen from room temperature to 37°.

The stabilizing effect of urea complexes is a consequence of the fact that autoxidation of unsaturated acids or esters is a chain reaction. In the crystalline matrix of the complex, contact of donor and acceptor molecules to propagate a chain is prevented, and the chain reaction mechanism cannot operate. Also, the urea probably offers a physical barrier against the free entry of oxygen.

The usual precautions against autoxidation are considerably reduced when easily autoxidizable substances are stored as urea complexes. The adducts are in an ideal form for storage and the included component can be obtained as needed by destruction of the complex by addition of water. Also, the urea complexes themselves have been used directly for the preparation of feeds in nutritional studies on essential fatty acids where urea does not interfere (5, 159). In addition, the urea complex of linoleic acid is reported to have some advantage in the sparing effect on Vitamin A (159).

It is noteworthy that other complex formers are able to stabilize autoxidizable materials by inclusion. Schlenk, Sand and Tillotson (160) have shown that the complexes of α- and β-dextrins and of desoxycholic acid with linoleic acid, methyl linolenate, Vitamin A palmitate and cinnamaldehyde are very resistant to autoxidation.

MISCELLANEOUS

Separation of Racemic Mixtures: This is a novel application of urea complexes described by Schlenk (161), and only a limited amount of information is available on it. Although it has been discussed in connection with substituted straight chain compounds and amino acids, its application to the separation of fat-derived racemic mixtures is readily apparent.

If a urea complex is prepared from any substance, the crystals will have either an all right-hand or an all left-hand helix. This is determined solely by chance, the first crystals to be formed acting as seed for all the others. If a urea complex is formed from a racemic mixture, crystals will be obtained which can

symbolically represented, assuming a right-hand helix forms, as D-form-right hand helix and L-form right-hand helix. These, of course, are no longer mirror images and should now have slightly different solubilities.

If a racemic mixture is treated with insufficient urea to precipitate the compound completely as complex, the isomer which preferentially precipitates will be the one whose complex has the lower solubility, and a slight concentration of it will be accomplished. A few crystals of this are reserved; the remaining crystals are decomposed, and the regenerated compound is again treated with insufficient urea to precipitate all the material but the solution is first seeded with the crystals with the right-hand helix which have been reserved for the purpose. This ensures that the same direction of helix will form as in the first precipitation, and an additional concentration of the same isomer will be achieved. This process, repeated many times should ultimately separate optical isomers. Racemic 2-chloro-octane has been separated in this way (161, 162).

In a modification of this procedure, Schlenk (163) has claimed to improve the separation of racemic mixtures by inoculating the reaction solution with insoluble solid organic substances which contain an asymmetric surface. Thus, 8 grams of racemic 2-butyl caprate was mixed with 100 grams of a saturated solution of urea in methanol and 1 gram of starch was added. The complex which forms was separated, decomposed and the included 2-butyl caprate separated. It weighed 1.4 grams and had α_D^{20} 1.17°.

Vinyl Esters: Vinyl esters of fatty acids readily form urea complexes in good to excellent yields (56-99%) (23, 114). Swern and Port (164) were able to separate vinyl pelargonate from difficult-to-remove crosslinking contaminants by precipitating the former as a complex leaving the impurities in the noncomplexing filtrate. Also, it was possible to recover vinly palmitate of suitable polymerization quality from complicated mixtures containing the monomer, polymers, inhibitors and other unknown impurities.

rea Complex Separations By Liquid-Solid Countercurrent Distribution: Summerwell (165) has reported an ingenious separation technique in which the reaction of fatty acids with urea to form complexes is used as the basis of a liquid-solid countercurrent distribution method of separation. The fatty acids and urea, dissolved in an appropriate solvent, serve as the moving liquid phase while the precipitated reaction products serve as the stationary solid phase. The character of the distribution curve obtained for a given mixture of fatty acids depends on the differences in the distribution coefficients for the individual fatty acids when they are distributed between solid inclusion compounds and organic solvent.

The method was shown to be effective in separating such mixtures of fatty acids as arachidic, stearic, palmitic and oleic acids, and in the separation of a cis-trans pair, oleic and elaidic acids. The method was also applied to the separation of an easily autoxidized material, salmon egg oil fatty acids, iodine number 350, and also to the hydrogenated fatty acids.

Mehta and coworkers (166) applied the above separation technique to the fractionation of the fatty acids of Indian kenaf seed oil and of karanja (Pongamia glabra) oil.

Urea columns have also been used for separation purposes. Moreno and coworkers (167) fractionated fatty acids and esters by passing a solution of them through a column of urea plus silica or other nonreactive substance. Kern and coworkers (168) separated the linear polyester (obtained by the reaction of succinic acid with 1,6-hexanediol) into fractions of different molecular weights by passing dilute benzene solutions of the polyster through a column of urea. Since higher molecular weight polyesters form urea complexes more readily (longer straight chains) than the lower molecular weight materials do, they are retained on the upper part of the urea column. Fractions were obtained having molecular weights from about 20,000 to 40,000.

eparation of Naturally-Occurring Branched Fatty Acids: Nunn (169) employed urea complexes to separate straight chain (complex-forming) fatty acids from sterculic acid, ω-(2-n-octylcycloprop-1-enyl) octanoic acid. Mehta and Dabhade (170) and Moreno and coworkers (102) used urea in the same fashion to concentrate chaulmoogra oil fatty acids (noncomplexing).

The urea complex separation technique is an excellent preliminary concentration step in working with the mixed fatty acids of new or relatively little known fats and oils. In a single, rapid and usually nondestructive step it permits removal of the straight chain acids on a scale from micro to any size laboratory scale. Both the complex and noncomplex fractions are available for further purification and/or detailed analysis.

Detection of Adulteration: Adulteration of butterfat has been detected by determining the yield and composition of the urea complexes obtained in fractionating the mixed fatty acids of the fat (171, 172). The adulteration of mustard oil with linseed and peanut oils has been detected similarly except that in this case the erucic acid is concentrated (173).

Other Complex-Formers: Barker and Ranano (174) have shown that certain liquid or soft and waxy surface active agents (polyoxyethylene esters of straight chain fatty acids containing 12-15 carbon atoms in the acyl group) set up to hard solids when mixed with urea. In one illustration, 70 parts of finely divided or molten urea mixed with 30 parts of a fatty acid ester of polyoxyethylene produces a plastic moldable mass which sets to a hard cake.

Potassium soaps of fatty acids are also reported to form urea complexes. Martinez Moreno and coworkers (175) and Bru and coworkers (176) prepared urea complexes of potassium soaps of palmitic, oleic and linoleic acids, and studied their x-ray diffraction characteristics. The palmitate and oleate complexes were known to have a mole ratio of urea to soap of 15 : 1 and 14.4 : 1, respectively, values corresponding to weight ratios of 3 : 1 and 2.7 : 1.

Certain types of quaternary ammonium compounds, such as di(dodecyl) dimethyl ammonium bromide, are also reported to form urea complexes (177). The products are free flowing powders in which the activity is proportional to the content of the quaternary compound.

Lecithin is reported to form an unstable addition complex with urea (178) but it is unlikely that it is a complex of the inclusion type. Finally, synthetic detergents which contain 0.5-5% by weight of a urea complex of a high molecular weight alcohol are reported to show less tendency to cause skin irritation (179).

X-Ray Diffraction Studies On Urea Complexes: Nicolaides and coworkers (180) have reported a method for distinguishing cis and trans isomers by x-ray diffraction measurements on single crystals of the corresponding urea complexes. These investigators prepared urea complexes from numerous fatty acids and esters and showed that an isolated trans double bond, as in elaidic acid for example, results in a shortening of the molecule by about 0.15 Å (using stearic acid as the standard for comparison), whereas a cis double bond results in a shortening of about 0.9 Å. The method is applicable to mono- and to some polyunsaturated substances and, to some extent, to mixtures. By this technique, these authors concluded that natural squalene is all trans.

In an extension of this work, Nicolaides and coworkers (181) described a method for locating a substituent in a hydrocarbon chain. By examining the intensity sequence of the x-ray patterns of urea complexes of twelve ketopalmitic and six hydroxypalmitic acids, good agreement was obtained between calculated and observed intensities. The "guest" molecule behaves as a one-dimensional crystal along the channel direction of the "host" structure.

Infrared Absorption Spectra of Urea Complexes: Several investigators have determined the infrared absorption spectra of urea complexes. Stuart (182) studied the N-H stretching frequencies of the urea-hexadecane complex with the aid of polarized

ıfrared radiation. Spectral differences were noted between urea and the complex. Stuart concluded that hydrogen bonding plays a predominant part and may well account for the spectral differences.

Illuminati and coworkers (183) studied the N-H stretching frequencies of urea complexes of paraffins, alcohols, monocarboxylic and dicarboxylic acids, esters, diamines and polymethylene halides. The complexes of paraffins, alcohols, monocarboxylic acids and esters form normal complexes with shifts of the asymmetric N-H vibration to longer wave lengths by 43 and 72 $cm.^{-1}$, and symmetric N-H vibration by 125 $cm.^{-1}$. The complexes of dicarboxylic acids, diamines, and polymethylene halides exhibit different N-H absorptions and thus must have different structures.

Barlow and Corish (184) used potassium chloride discs of complexes of paraffins, bromides, alcohols, acids and esters. They concluded on the basis of differences between the infrared spectra of urea and of the complexes that one can determine whether a complex has formed. The conclusions of earlier investigators (183) was largely corroborated.

Mecke and Kutzelnigg (185) also studied the urea complexes of hydrocarbons, alcohols, ketons, acids, esters, nitriles and halogenated hydrocarbons in the 1-25 micron region. They showed that infrared spectra are useful in identifying complexes and that potassium bromide pellets, mulls or suspensions can be used, depending on the included compound.

Other Physical Studies of Complexes: Meakins (186) reported that the large dielectric absorption of urea complexes at microwave frequencies is due to the orientation of the long-chain dipoles in the urea crystal lattice. Ferroni (187) studied films of the urea complex of palmitic acid and reported that the complex can exist in unimolecular layers.

TABLE I

EFFECT OF POSITION AND NUMBER OF BRANCHES AND/OR RINGS ON THE UREA COMPLEX-FORMING ABILITY OF ESTERS (5, 7, 17)

COMPLEX-FORMING[a]	NONCOMPLEX-FORMING
RINGS IN MOLECULE	
PHENYL DODECANOATE	PHENYL HEXANOATE
PHENYL OCTANOATE (BORDERLINE)	
BENZYL DODECANOATE	BENZYL OCTANOATE
BENZYL DECANOATE	
CYCLOHEXYL DODECANOATE	CYCLOHEXYL OCTANOATE
	CYCLOHEXYL DECANOATE
DODECYL PHENYLACETATE	CHOLESTERYL ACETATE
DECYL PHENYLACETATE	CHOLESTERYL OCTANOATE
	CHOLESTERYL PALMITATE
β-NAPHTHYL PALMITATE	
	n-OCTADECYL BENZOATE
HYDROQUINONE DIHEXANOATE	
HYDROQUINONE DIBUTYRATE	METHYL 9-PHENYLSTEARATE
BRANCHES IN MOLECULE	
-METHYLBUTYL DECANOATE	2-METHYLBUTYL PROPIONATE
2-METHYLBUTYL DODECANOATE	2-METHYLBUTYL OCTANOATE
3-METHYLBUTYL HEXANOATE	3-METHYLBUTYL PROPIONATE
3-METHYLBUTYL OCTANOATE	
2-ETHYLBUTYL DECANOATE	2-ETHYLBUTYL OCTANOATE
2-ETHYLBUTYL DODECANOATE	
1-METHYLHEPTYL BUTYRATE	1-METHYLHEPTYL ACETATE
	1-METHYLHEPTYL PROPIONATE
2-ETHYLHEXYL OCTANOATE	2-ETHYLHEXYL HEXANOATE
2-ETHYLHEXYL DODECANOATE	
CITRONELLYL OCTANOATE	CITRONELLYL BUTYRATE
GERANYL DODECANOATE	GERANYL OCTANOATE
METHYL 9,10-DIHYDROXYSTEARATES	
ISOPROPYL OLEATE	ISOPROPYL 12-METHYLTETRADE-CANOATE
ISOPROPYL 11-EICOSENOATE	
ISOPROPYL 13-DOCOSENOATE	
METHYL 12-METHYLTETRADECANOATE	3,9-DIETHYL-6-TRIDECYL PROPIONATE
METHYL 13-METHYLPENTADECANOATE	3,9-DIETHYL-6-TRIDECYL NONANOATE
HYL 3-METHYLUNDECANOATE	
12-METHYLTETRADECYL ACETATE	

a) If the straight-chain portion of a molecule is longer than that of any tabulated compound it will also form a complex. If the straight chain is shorter, it should be assumed that complex formation is unlikely.

HEATS OF UREA COMPLEX FORMATION AND EQUILIBRIUM CONSTANTS (a)

COMPOUND	HEAT OF FORMATION, ΔH, KCAL./MOLE: CALORIMETRIC (7a,18)	HEAT OF FORMATION, ΔH, KCAL./MOLE: FROM EQUILIBRIUM CONSTANT (7a,30)	EQUILIBRIUM CONSTANT, K AT 25° (30,31)	STABILITY CONSTANTS, $\frac{1}{K}$
FATTY ACIDS				
BUTYRIC		5.6	0.33	3.0
VALERIC	8.5	----		
CAPROIC	----	----	0.163	6.1
HEPTANOIC	11.5	----	----	----
CAPRYLIC	13.2	10.0	0.052;0.07	14;19
PELARGONIC	----	----	0.034	29
CAPRIC	17.0	17.8	0.0185	54
LAURIC	21.0	20.1	0.00634	157
UNDECYLENIC	----	19.7	----	----
MYRISTIC	22.5	22.5;24.5	0.0029; 0.00357	280,344
PALMITIC	26.6	26.7	0.0017	590
STEARIC	29.5	29.0	0.00020	5,000
OLEIC	----	27.4	0.0021	480
ELAIDIC	----	----	0.0015	666
LINOLEIC	----	----	0.019	53
METHYL ESTERS				
UNDECYLENATE	----	17.4	----	----
MYRISTATE	16.1	----	----	----
PALMITATE	19.4	----	----	----
STEARATE	22.2	19.0	0.0001	10,000
OLEATE	20.6	25.0	0.0019	530

COMPOUND	HEAT OF FORMATION, ΔH, KCAL./MOLE CALORIMETRIC(7a,18)	FROM EQUILIBRIUM CONSTANT (7a,30)	EQUILIBRIUM CONSTANT, K AT 25°(30,31)	STABILITY CONSTANTS, $\frac{1}{K}$
METHYL ESTERS				
LINOLEATE	----	24.6	0.023	44
LINOLENATE	----	22.5	0.66	1.5
11-EICOSENOATE	21.2	----	----	----
13-DOCOSENOATE	23.7	----	----	----
12-METHYLTETRADECANOATE	7.8	----	----	----
ISOPROPYL ESTERS				
OLEATE	7.0	----	----	----
11-EICOSENOATE	15.5	----	----	----
13-DOCOSENOATE	19.5	----	----	----
n-ALCOHOLS				
1-HEXANOL	2.6	----	----	----
1-HEPTANOL	----	1.5	----	----
1-OCTANOL	5.4	----	----	----
1-DECANOL	8.4	8.4	----	----
1-DODECANOL	11.7	11.9	----	----
1-TETRADECANOL	14.3	14.6	----	----
1-PENTADECANOL	16.2	17.0	----	----
1-HEXADECANOL	18.0	18.2	----	----
1-HEPTADECANOL	19.7	20.1	----	----
1-OCTADECANOL	20.7	20.9	----	----

(a) For the process: Complex = Reactant + m Urea

TABLE III

COMPOSITION OF UREA COMPLEXES OF FATTY COMPOUNDS AND THEIR DISSOCIATION TEMPERATURES (5, 7, 43, 44)

SATURATED AND SUBSTITUTED FATTY ACIDS	RATIO OF UREA TO ACID		DISSOCIATION TEMP.°C	UNSATURATED FATTY ACIDS	RATIO OF UREA TO ACID		DISSOCIATION TEMP.°C
	MOLAR	WEIGHT			MOLAR	WEIGHT	
BUTYRIC	4.0	2.7	a	10-HENDECENOIC	9.2	3.0	90
VALERIC	4.6	2.7	a	OLEIC	13.7	2.9	110
CAPROIC	5.5	2.8	64	PETROSELAIDIC	14.1	3.0	a
HEPTANOIC	6.0	2.8	a	RICINOLEIC	14.3	2.9	a
CAPRYLIC	6.7	2.8	73	LINOLEIC	13.1	2.8	a
PELARGONIC	7.6	2.9	80.5	10,12-LINOLEIC	14.2	3.0	a
CAPRIC	8.2	2.9	85	LINOLENIC	13.6	3.0	a
UNDECANOIC	8.9	2.9	a	α-ELEOSTEARIC	13.7	3.0	a
LAURIC	10.0	3.0	92.5	β-ELEOSTEARIC	14.0	3.0	a
TRIDECANOIC	11.8	3.3	96	pseudo-ELEOSTEARIC	13.7	3.0	a
ISTIC	11.6	3.0	103	ERUCIC	16.8	3.0	a
PALMITIC	12.8	3.0	114	BRASSIDIC	16.8	3.0	a
STEARIC	14.2	3.0	126				
ARACHIDIC	16.0	3.1	a				
BEHENIC	16.7	2.9	a				
CIS-9,10-EPOXYSTEARIC	13.4	2.7	118				
TRANS-9,10-EPOXYSTEARIC	13.4	2.7	125				
9,10-DIHYDROXY-STEARIC, M.P.95°	14.7	2.8	107				
12-HYDROXY-STEARIC	14.0	2.8	125				
12-KETOSTEARIC	14.4	2.9	115				

a Not available

TABLE III (continued)

METHYL ESTERS	RATIO OF UREA TO ESTER MOLAR	RATIO OF UREA TO ESTER WEIGHT	DISSOCIATION TEMP.°C	ALCOHOLS	RATIO OF UREA TO ALCOHOL MOLAR	RATIO OF UREA TO ALCOHOL WEIGHT	DISSOCIATION TEMP.°C
CAPRYLATE	6.7	3.3	55	OCTANOL	6.9	3.2	a
CAPRATE	9.6	3.1	67	NONANOL	a	a	57
LAURATE	11.1	3.1	77.5	DECANOL	a	a	68
MYRISTATE	12.1	3.0	96	DODECANOL	9.6	3.1	80
PALMITATE	13.5	3.0	118	TETRADECANOL	11.1	3.1	91
STEARATE	14.8	3.0	132	HEXADECANOL	12.1	3.0	107
OLEATE	14.5	3.0	110	OCTADECANOL	13.5	3.0	124
ELAIDATE	13.9	2.8	125	OLEYL	14.3	3.2	98
LINOLEATE	14.2	3.0	a	ELAIDYL	14.3	3.2	118
OLENATE	13.7	3.0	a				
9,10-DIHYDROXY-STEARATE,M.P.70°	14.9	2.7	120				
9,10-DIHYDROXY-STEARATE,M.P.103°	14.9	2.7	114				

a Not available

TABLE III (continued)

MISCELLANEOUS COMPOUNDS	RATIO OF UREA TO COMPOUND		DISSOCIATION TEMP. °C
	MOLAR	WEIGHT	
VINYL PELARGONATE	10.2	3.3	52
VINYL LAURATE	a	a	79
VINYL PALMITATE	15.1	3.2	113
VINYL OCTADECYL ETHER	13.9	2.8	125.5
1-MONOCAPRYLIN	9.4	2.6	74
1-MONOCAPRIN	11.0	2.7	86
1-MONOLAURIN	12.1	2.7	97
1-MONOMYRISTIN	14.4	2.9	109
1-MONOPALMITIN	16.5	3.0	118
1-MONOSTEARIN	17.4	2.9	129

a Not available

TABLE IV

ENRICHMENT PROCEDURES. SEGREGATION OF MIXED FATTY ACIDS, ESTERS AND OTHER FAT DERIVATIVES INTO LOW AND HIGH IODINE NUMBER (I.N.) FRACTIONS

STARTING MATERIALS	ISOLATED FROM COMPLEX %(a)	ISOLATED FROM COMPLEX I.N.	ISOLATED FROM FILTRATE %(a)	ISOLATED FROM FILTRATE I.N.	REFERENCES
Coconut Oil Fatty Acids, I.N. 11	63	10	31	16	83
Chinese Tallow Fatty Acids, I.N. 19	53	6.5	36	38	62
Castor Oil Fatty Acids, I.N. 74	19	20	78	90	83
Olive Oil Fatty Acids, I.N. 81	26	46	75	85.5	62
Peanut Oil Fatty Acids, I.N. 84	82	80	13	161	83
Mustardseed Oil Fatty Acids, I.N. 91	81	80	14	187	83
Rice Oil Fatty Acids, I.N. 99	21	29	76	116	142
	36	40	60	135	
	54	57	42	159	
Nitriles From Mixed Fatty Acids, I.N. 107	33	62	64	134	82, 141
Cottonseed Oil Fatty Acids, I.N. 113	45	62	55	144	76
Corn Oil Fatty Acids, I.N. 120	43	76	57	154	62, 82, 141
I.N. 126	42	81	52	164	
Salmon Oil Fatty Acids, I.N. 128	30	53	70	160	82, 141
Seal Oil Fatty Acids, I.N. 129	43/(b)	68	49	211	143
Soybean Oil Fatty Acids, I.N. 132	40-50	77-90	58-47	169-177	82, 141
I.N. 141	9	56	81	162	62
	37	88	56	180	
	67	119	27	191	
Methyl Esters of Soybean Oil Fatty Acids, I.N. 139	42	97	52	175	82, 141
I.N. 141	22	78	70	160	82, 141
Herring Oil Fatty Acids, I.N. 136	56/(b)	58	42	246	143
Shark Liver Oil Fatty Acids, I.N. 138	80	121	17	243	144
Safflowerseed Oil Fatty Acids, I.N. 143	34	50	63	174	83

TABLE IV (continued)

STARTING MATERIALS	ISOLATED FROM COMPLEX %(a)	ISOLATED FROM COMPLEX I.N.	ISOLATED FROM FILTRATE %(a)	ISOLATED FROM FILTRATE I.N.	REFERENCES
Salmon Head and Viscera Oil Fatty Acids, I.N. 145	47	65	34	242	143
Gourdseed Oil Fatty Acids, I.N.148	29	81	68	160	62
Tuna Body Oil Fatty Acids, I.N.150	40/ (b)	42	50	243	143
Menhaden Oil Fatty Acids, I.N.160	52/ (b)	30	48	255	143
Tall Oil Fatty Acids, I.N.167	12	63	83	182	82, 141
Linseed Oil Fatty Acids, I.N.169	40	109	58	204	82, 141
I.N.175	55	115	40	278	
I.N.180	16	54	77	199	82,141
Methyl Esters of Linseed Oil Fatty Acids, I.N. 187	35	130	50	224	82, 141
Alcohols From Reduction of Linseed Oil, I.N. 196	28	106	63	233	82, 141
Pilchard Oil Fatty Acids, I.N.201	77	150	22	386	145, 146, 147
Alcohols From Reduction of Pilchard Oil, I.N. 218	70	155	30	365	145
Salmon Egg Oil Fatty Acids, I.N.207	50	81	49	290	143
Fatty Acids of Beef Testicular Tissue, I.N. 313	51	300	20	416	148

(a) Operating losses account for the fact that the sum of the yields of products isolated from the complex and noncomplex fractions is less than 100%.

(b) The figure given is for the total quantity of acids complexed down to -30° even though the operation was conducted stepwise.

TABLE V

EFFECT OF DIFFERENT RATIOS OF UREA TO MENHADEN OIL FATTY ACIDS, IODINE NUMBER, 159.5, ON YIELD AND IODINE NUMBER OF FRACTIONS (143)

MOLE RATIO OF UREA TO ACIDS	ISOLATED FROM COMPLEX		ISOLATED FROM FILTRATE	
	%	I. N.	%	I. N.
4.6 : 1	12	13	81	193
9.1 : 1	30	22	62	243
13.8 : 1	49	48	42	308
18.4 : 1	61	55	36	331
23.0 : 1	63	73	34	342

TABLE VI

ENRICHMENT OF POLYUNSATURATED ACIDS FROM FISH OIL SOURCES AT 5° (149)

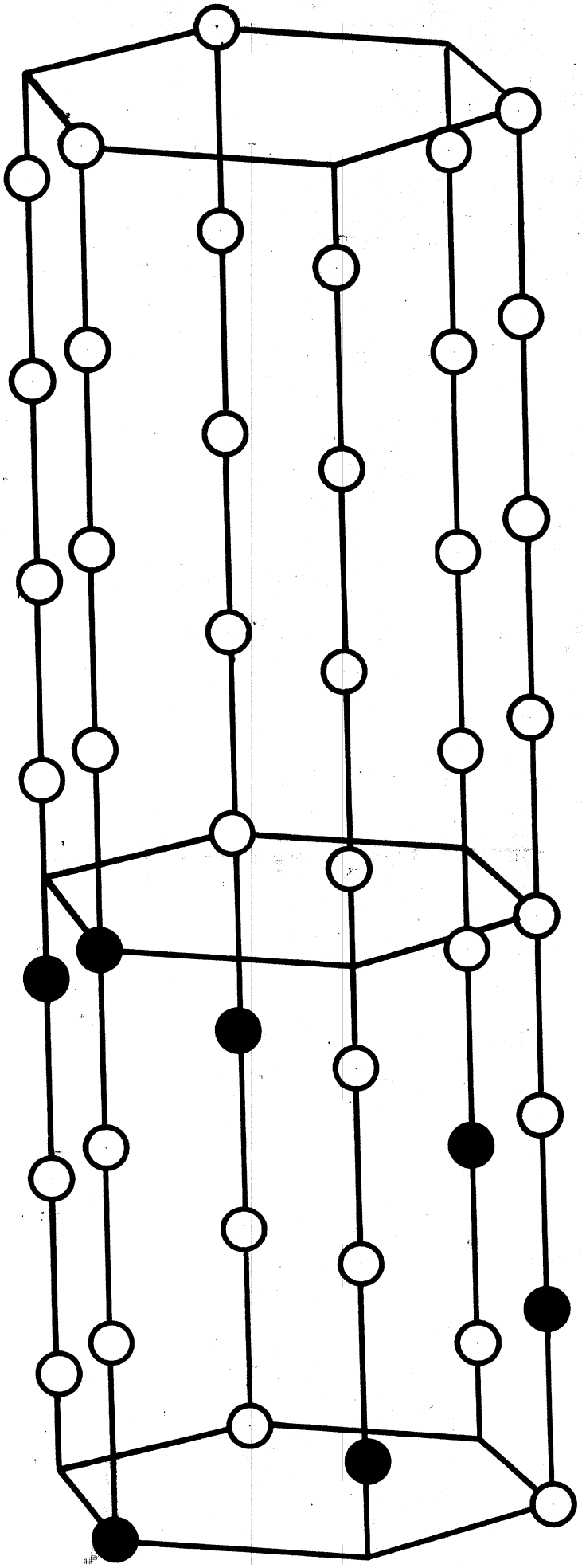
OIL	IODINE NUMBER OF STARTING FATTY ACIDS	IODINE NUMBER OF FATTY ACIDS FROM FILTRATE (NONCOMPLEX)
Cod Liver	159	350
Shark Liver	140	327
Pollack Liver	156	322
Herring	148	294
Salmon	151	326
Industrial Shark Liver	122	313
Menhaden	167	356

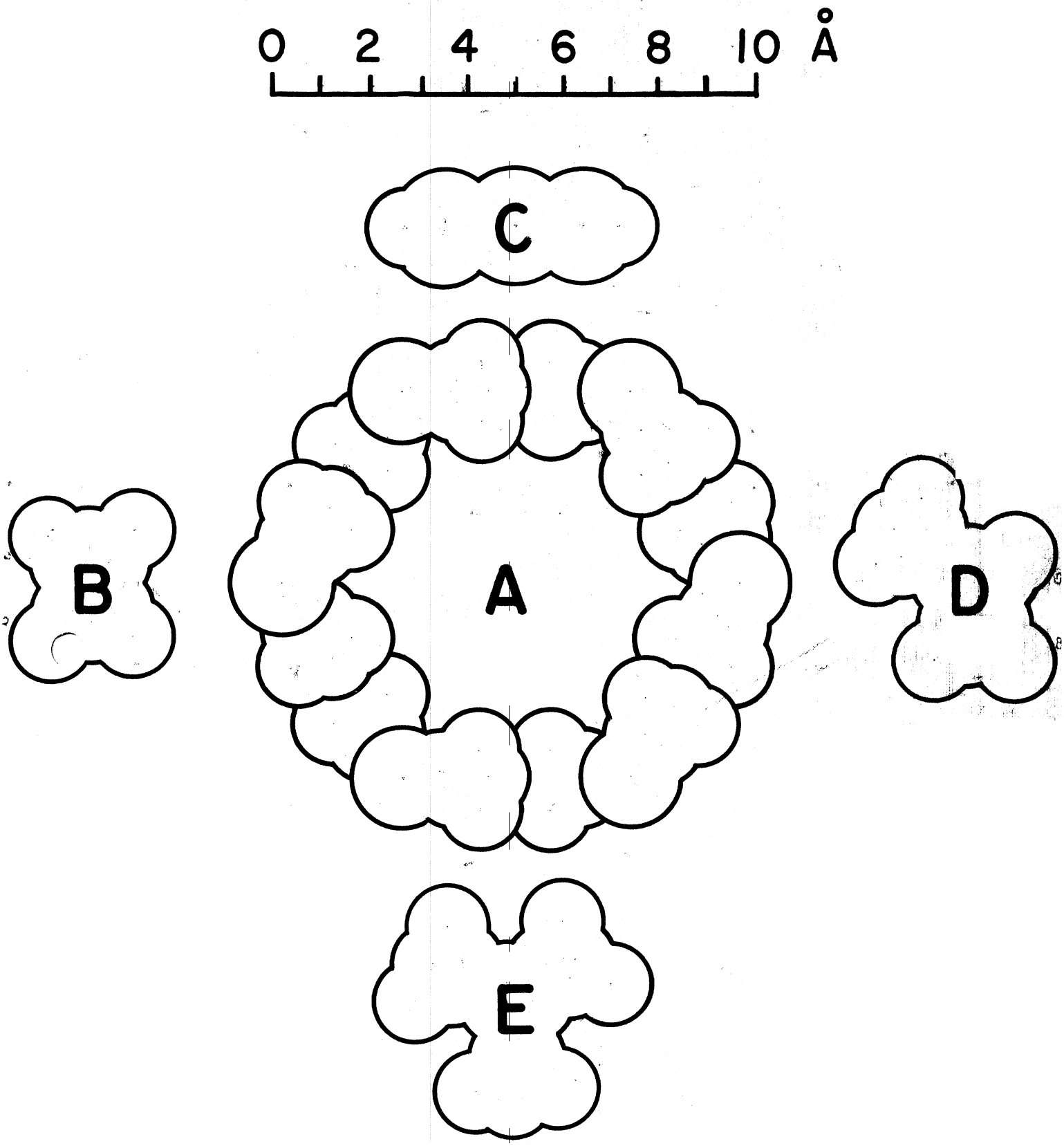
LEGEND FOR FIGURES

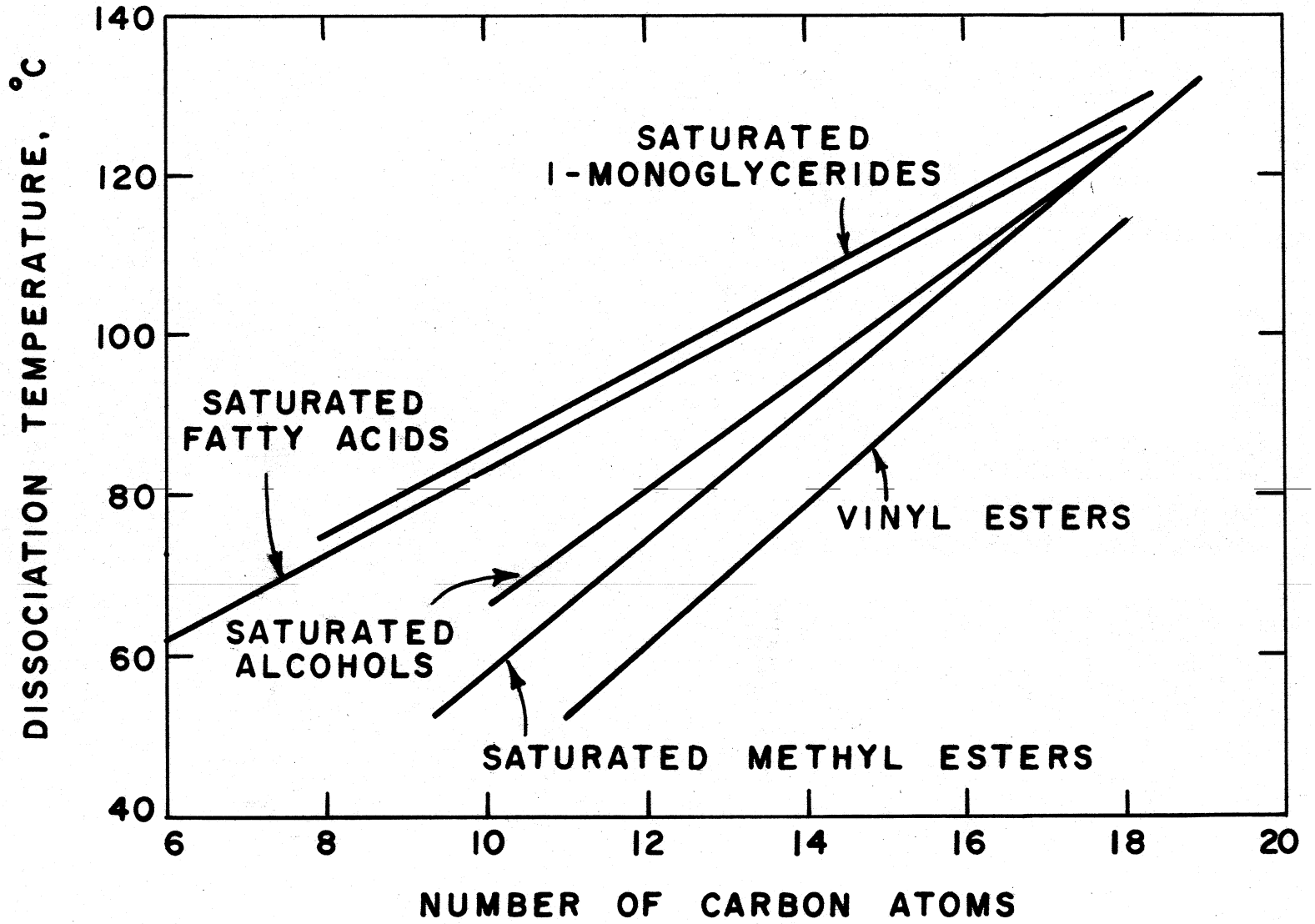
FIGURE 1 : STRUCTURE OF HEXAGONAL UREA IN UREA COMPLEXES. (SOLID CIRCLES ARE UREA MOLECULES)

FIGURE 2 : CROSS SECTION OF HEXAGONAL UREA (A); n-OCTANE (B); BENZENE (C); 3-METHYLHEPTANE (D); 2,2,4-TRIMETHYLPENTANE (E)

FIGURE 3 : DISSOCIATION TEMPERATURE OF UREA COMPLEXES AGAINST NUMBER OF CARBON ATOMS IN INCLUDED COMPOUND




0 2 4 6 8 10 Å
C
B
A
D
E


DISSOCIATION TEMPERATURE, °C
140
120
100
80
60
40
6
8
10
12
14
16
18
20
NUMBER OF CARBON ATOMS
SATURATED
1-MONOGLYCERIDES
SATURATED
FATTY ACIDS
SATURATED
ALCOHOLS
VINYL ESTERS
SATURATED METHYL ESTERS

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